SCOTTISH HOSPITALS INQUIRY

Bundle of documents for Oral hearings commencing from 19 August 2024 in relation to the Queen Elizabeth University Hospital and the Royal Hospital for Children, Glasgow

Bundle 27 – Volume 15
Miscellaneous Documents

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Table of Contents

1.	A50578926	Commodity Action Report & Eps Bulletin (CAREB) - Authorising Engineers - Extension - NP813-13 - 29 April 2016	Page 10
2.	A50578957	Statutory Compliance Audit Reporting Tool (SCART) - Water Question Set – 2014	Page 17
3.	A50381800	UK Standards for Microbiology Investigations - Investigation of superficial mouth samples, UKHSA, B4 - Issue 7.2 - 23 August 2022	Page 20
4.	A50383181	UK Standards for Microbiology Investigations - Review of Users' comments received by Working Group for Microbiology Standards in Clinical Bacteriology - Investigation of superficial mouth samples, PHE, B4 - Issue 1 - 20 October 2015	Page 40
5.	A50383299	UK Standards for Microbiology Investigations - Investigation of samples from paranasal sinuses, UKHSA, B5 - Issue 8 - 16 September 2024	Page 49
6.	A50383431	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology - Investigation of samples from paranasal sinuses, UKHSA, B5 - Issue 1 - 16 September 2024	Page 78
7.	A50383651	UK Standards for Microbiology Investigations - Investigation of whooping cough, PHE, B6 - Issue 9 - 11 May 2018	Page 84

8. A50383862 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of Whooping Cough, PHE, B6 - Issue 2 - 11 May 2018 Page 107

9. A50386188 UK Standards for Microbiology Investigations - Investigation of Throat Related Specimens, PHE, B9 - Issue 9 - 15 April 2015 Page 111

10. A50386196 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of Throat Related Specimens, PHE, B9 - Issue 2 - 15 April 2015 Page 140

11. A50386190 UK Standards for Microbiology Investigations - Processing of Faeces for Clostridium difficile, PHE, B10 - Issue 1.7 - 19 September 2018 Page 146

12. A50386189 UK Standards for Microbiology Investigations - Investigation of swabs and superficial soft tissue infections, PHE, B11 - Issue 6.5 - 19 December 2018 Page 170

13. A50386192 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of swabs from skin and superficial soft tissue infections, PHE, B11 - Issue 2 - 04 May 2016 Page 207

14. A50386191 UK Standards for Microbiology Investigations - Investigation of pus and exudates, PHE, B14 - Issue 6.2 - 22 November 2016 Page 216

15. A50386193 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of pus and exudates, PHE, B14 - Issue 2 - 20 June 2016 Page 250

16. A50386195 UK Standards for Microbiology Investigations - Investigation of bile, PHE, B15 - Issue 7 - 11 January 2018 Page 258

17.	A50386194	UK Standards for Microbiology Investigations - Investigation of bile, PHE, B15 - Issue 2 - 11 January 2018	Page 279
18.	A50387379	UK Standards for Microbiology Investigations - Investigation of tissues and biopsies from deep-seated sited and organs, PHE, B17 - Issue 6.3 - 05 January 2018	Page 284
19.	A50387378	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of tissues and biopsies from deep-seated sites and organs, PHE, B17 - Issue 1 - 08 April 2016	Page 310
20.	A50387382	UK Standards for Microbiology Investigations - Investigation of intravascular cannulae and associated specimens, PHE, B20 - Issue 6.1 - 27 June 2017	Page 319
21.	A50387380	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of tissues and biopsies from deep-seated sites and organs, PHE, B20 - Issue 1 - 30 November 2015	Page 343
22.	A50387381	UK Standards for Microbiology Investigations - Investigation of Cerebrospinal Fluid Shunts, PHE, B22 - Issue 6.1 - 12 June 2015	Page 348
23.	A50387385	UK Standards for Microbiology Investigations - Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology - Investigation of Cerebrospinal Fluid Shunts, PHE, B22 - Issue 1 - 18 May 2015	Page 368
24.	A50387383	UK Standards for Microbiology Investigations - Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid, PHE, B25 - Issue 6 - 20 February 2015	Page 371

25.	A50387384	UK Standards for Microbiology Investigations - Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology - Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid, PHE, B25 - Issue 1 - 20 February 2015	Page 392
26.	A50387386	UK Standards for Microbiology Investigations - Investigation of Fluids from Normally Sterile Sites, PHE, B26 - Issue 6.2 - 03 October 2018	Page 394
27.	A50387387	UK Standards for Microbiology Investigations - review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology , PHE, B26 - Issue 1 - 15 June 2015	Page 418
28.	A50387388	UK Standards for Microbiology Investigations - Investigation of Cerebrospinal Fluid, PHE, B27 - Issue 6.1 - 31 May 2017	Page 426
29.	A50387390	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of Cerebrospinal Fluid, PHE, B27 - Issue 1 - 24 February 2015	Page 455
30.	A50387391	UK Standards for Microbiology Investigations - Investigation of Genital Tract and Associated Specimens, PHE, B28 - Issue 4.6 - 26 April 2017	Page 468
31.	A50387393	UK Standards for Microbiology Investigations - Investigation of specimens for screening for MRSA, PHE, B29 - Issue 7 - 26 May 2020	Page 508
32.	A50387394	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for Microbiology standards in clinical bacteriology - Investigation of specimens for screening for MRSA, PHE, B29 - Issue 1 - 26 May 2020	Page 526
33.	A50387395	UK Standards for Microbiology Investigations - Investigation of specimens other than blood for parasites, PHE, B31 - Issue 5.1 - 28 June 2017	Page 533

34.	A50387392	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of specimens other than blood for parasites, PHE, B31 - Issue 2 - 01 March 2017	Page 593
35.	A50417932	UK Standards for Microbiology Investigations - Investigation of Bone Marrow, PHE, B38 - Issue 2 - 12 October 2015	Page 599
36.	A50417936	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of bone marrow, PHE, B38 - Issue 1 - 12 October 2015	Page 621
37.	A50417941	UK Standards for Microbiology Investigations - Investigation of Dermatological Specimens and Superficial Mycoses, PHE, B39 - Issue 3.1 - 29 December 2016	Page 624
38.	A50417944	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of Dermatological Specimens for Superficial Mycoses, PHE, B39 - Issue 2 - 11 August 2015	Page 650
39.	A50417946	UK Standards for Microbiology Investigations - Investigation of specimens for Mycobacterium species, PHE, B40 - Issue 7.3 - 05 October 2020	Page 653
40.	A50417948	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of specimens for Mycobacterium species, PHE, B40 - Issue 2 - 10 January 2017	Page 709
41.	A50417959	UK Standards for Microbiology Investigations - Investigation of urine, PHE, B41 - Issue 8.7 - 11 January 2019	Page 721

42. A50417964 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of urine, PHE, B41 - Issue 1 - 15 August 2016 Page 772

43. A50417971 UK Standards for Microbiology Investigations - Investigation of bone and soft tissue associate d with osteomyelitis, PHE, B42 - Issue 2 - 14 December 2015 Page 783

44. A50417979 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of bone and soft tissue associated with osteomyelitis, PHE, B42 - Issue 1 - 14 December 2015 Page 811

45. A50417984 UK Standards for Microbiology Investigations - Investigation of orthopaedic implant associated infections, PHE, B44 - Issue 2.1 - 18 August 2021 Page 816

46. A50417990 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of orthopaedic implant associated infections, PHE, B44 - Issue 1 - 23 February 2016 Page 850

47. A50419804 UK Standards for Microbiology Investigations - Screening for Neisseria meningitidis, PHE, B51 - Issue 2 - 12 March 2014 Page 864

48. A50419807 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Screening for Neisseria meningitidis, PHE, B51 - Issue 1.1 - 12 March 2014 Page 882

49. A50419817 UK Standards for Microbiology Investigations - Investigation of infectious causes of dyspepsia, PHE, B55, Issue 7 - 03 October 2019 Page 887

50. A50419823 UK Standards for Microbiology Investigations - review of user's comments received by Working group for microbiology standards in clinical bacteriology - Investigation of Infectious causes of dyspepsia, PHE, B55 - Issue 2 - 03 October 2019 Page 903

51. A50419828 UK Standards for Microbiology Investigations - Investigation of bronchoalveolar lavage, sputum and associated specimens, PHE, B57 - Issue 3.5 - 08 May 2019 Page 906

52. A50419833 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Investigation of bronchoalveolar lavage, sputum and associated specimens, PHE, B57 - Issue 1 - 02 October 2015 Page 944

53. A50419843 UK Standards for Microbiology Investigations - Detection of Carriage of group B Streptococci, PHE, B58 - Issue 3.1 - 26 June 2018 Page 956

54. A50419892 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Detection of Carriage of group B Streptococci, PHE, B58 - Issue 1 - 19 June 2015 Page 980

55. A50419901 UK Standards for Microbiology Investigations - Detection of Enterobacteriaceae producing extended spectrum β-lactamases, PHE, B59 - Issue 4.1 - 17 August 2016 Page 987

56. A50419969 UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Detection of Enterobacteriaceae producing extended spectrum β-lactamases, PHE, B59 - Issue 1 - 04 August 2016 Page 1018

57. A50419984 UK Standards for Microbiology Investigations - Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases), PHE, B60, Issue 3.1 - 01 June 2022 Page 1020

58.	A50419997	UK Standards for Microbiology Investigations - Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases), PHE, B60, Issue 3.1 - 01 June 2022	Page 1052
59.	A50420060	UK Standards for Microbiology Investigations - Investigation of specimens for ectoparasites, PHE, B61 - Issue 2.2 - 12 October 2018	Page 1084
60.	A50420070	UK Standards for Microbiology Investigations - Abdominal organ transport fluid testing, PHE, B62, Issue 1 - 29 January 2020	Page 1146
61.	A50420075	UK Standards for Microbiology Investigations - Review of users' comments received by Working Group for microbiology standards in clinical bacteriology - Abdominal organ transplant fluid testing, PHE, B62 - Issue 1 - 19 February 2020	Page 1158
62.	A36711569	NHSGGC, 'SOP Environmental Monitoring in the Haematology Oncology Unit Ward 6A', HPS, Version 1, Effective from October 2019	Page 1166
63.	A37852376	NHSGGC, 'SOP Environmental Monitoring in the Haematology Oncology Unit Ward 6A', HPS, Version 1, Effective from October 2019 - with comments from Ms. Annette Rankin	Page 1174
64.	A44944291	Environmental Monitoring SOP, LP089 - 17 March 2015	Page 1181
65.	A44944289	Environmental Monitoring SOP, LP539 - 08 March 2017	Page 1199

C A R E B

Commodity Action Report & Eps Bulletin

Authorising Engineers - Extension (NP813/13)

Prepared by:

Robert Armstrong
National Procurement
Commodity Manager

29th April 2016

Document Information

Contract Name:	Commodity Action Report & Eps Bulletin for Authorising Engineers			**Contract Reference:**	NP813/13		
National Spend:		**Savings Opportunity:**		**Start Date:**	16/10/2016	**Expiry Date:**	15/10/2017
Contract Manager	Robert Armstrong, Commodity Manager)	**Tel :**		**Email:**			

Awarded Supplier (see main text in original CAREB for details of selection and evaluation process) – Reason for Extension
The following suppliers have been awarded onto the Framework, details of the disciplines they have been awarded are provided in the original CAREB.

Atkins Ltd; Legionella Control International Ltd; BOC Healthcare, Sodexho Property Solutions Ltd; Hulley & Kirkwood Consulting Engineers Ltd; Turner Facilities Management Ltd

National Procurement advise that due to the specialised nature of this framework agreement, Health Facilities Scotland have agreed that the current framework agreement should be extended to help the Health Boards meet their statutory obligations to ensure safe systems of work and inspection regimes are in place and carried out.

Benefit Summary (See main text for full details)

Health Board	No Change Benefit	Optimum Benefit	Contract Extension		Specific HB Comment
			Delivered Saving	Remaining Benefit	
Ayrshire & Arran	£0				It is not practical to identify secured savings against each Health Board. The calculation of benefit is based upon the anticipated total number of inspections which will be carried per annum. Optimum benefits are based on Health Boards estimated usage of the contact detailed in Para 6 of the original CAREB.
Borders	£0				
Dumfries & Galloway	£0				
Fife	£0				
Forth Valley	£0				
Grampian	£0				
Greater Glasgow & Clyde	£0				
Lothian	£0				
NWTC (Golden Jubilee)	£0				
Shetlands	£0				
Tayside	£0				
Total	£0				

Contract Extension (see main text for further details)
The Framework has been extended for a further year. **The twelve month extension option has now been utilised extending the current framework until 15 October 2017.**

Table of Contents

1. **Commodity Description / Scope** 4
2. **Current Position / Contract Extension** 4
3. **Changes during Extension Period** 4
4. **Contract Value / Calculation of Benefits** 4
5. **Implementation** 5
6. **Next Steps** 5
7. **Further Information** 6

C A R E B National Procurement	Authorising Engineers - Extension	NP813/13

1. Commodity Description / Scope

There is a statutory requirement to ensure safe systems of work are in place. One way of ensuring this is to employ an Authorising Engineer to ensure that safe systems of work and inspection regimes are in place and carried out across the organisation. The Authorising Engineer does not perform any physical work on a site but reviews and approves method statements and ensures that the organisation's Authorising Persons are appropriately trained and qualified. The duties of Authorising Engineers within the Health Services have been clarified with the issuing of new Scottish Health Technical Memoranda. See Section 1.1 of original CAREB.

The scope of this contract is for the provision of Authorising Engineer services for NHS Scotland Health Organisations to supplement existing arrangements and in-house capability. The scope of services is restricted to five (5) disciplines as outlined in the original CAREB document.

The Authorising Engineer acts as an independent technical advisor in specialist disciplines in which statute requires that organisations have independent advice.

This extension to the current framework agreement will enable the Health Boards to meet their statutory obligations to ensure that safe systems of work and inspect regimes are in place and carried out.

2. Current Position / Contract Extension

The framework agreement for the provision of the services of Authorising Engineers is due to expire on 15th October 2016, with the option to extend a period of up to twelve (12) months.

The CAP has been approached and approved the proposal to extend this contract for a period of twelve (12) months making then new expiry date 15th October 2017.

This will allow Health Boards to access the current framework beyond 15th October 2016 subject to available funding.

It is anticipated that this service will be re-tendered prior to the end of the contract extension period.

3. Changes during Extension Period

All suppliers with the exception of Atkins Limited have agreed to support the framework during the extension period and will hold current pricing rates offered.

Atkins Limited did not respond to correspondence sent to them.

4. Contract Value / Calculation of Benefits

Based on recent uptake information, the total spend since the framework commenced is £385,632 as detailed below:

Health Board	Contract Spend for the period Oct 13 to Jan 16
Ayrshire & Arran	
Borders	
Dumfries & Galloway	
Fife	
Forth Valley	
Grampian	
Greater Glasgow & Clyde	
Lanarkshire	No reported spend
Lothian	
National Waiting Centre	
Orkney	
State Hospital	
Shetland	No reported spend
Tayside	
Western Isles	No reported spend
Total	

NHS Western Isles and NHS Shetland use a combination of in-house staff, SLA (via NHS Grampian) and local Authorising Engineers at a lower cost to provide these services, hence there is no spend listed against those Health Boards. NHS Lanarkshire use a mixture of contract and non-contract suppliers depending on the specific area of requirement.

5. Implementation

EPS Implementation Notes

CCM will be amended to reflect the extension period dates.

Contractual Terms

The original framework was awarded on National Procurement's standard terms and conditions of contract for the provision of services. Health Boards should ensure that all orders reference the NP813/13 Contract Reference on all purchase orders.

6. Next Steps

Health Boards will access the contract by assessment of needs and should review and identify requirements for the services of Authorising Engineers when appropriate.

National Procurement will distribute the CAREB extension to the NHS Energy and Natural Resources Group chaired by HFS and Heads of Estates throughout the Health Boards.

7. Further Information

Please refer to the original CAREB document for further information regarding the initial award.

Version History

Version. No.	Version. Date	Revised By	Description	Filename
1.0	01/11/13	D Taggart	CAREB – Contract Name	
1.1	29/04/16	R Armstrong	CAREB – Authorising Engineer - Extension	Z:\12 PC&F\Sourcing\Estates & Facilities\Contracts\NP813.13 Authorising Engineers\12 Recommendation\05 CAREB

Distribution

To	Action	
All HoP's	Local engagement with stakeholders to secure optimum value for money option	29/04/16
All HoE's	Local engagement with stakeholders to secure optimum value for money option	29/04/16
Strategic Sourcing	Load CAREB and other relevant data to intranet	29/04/16
HB Project Manager (e procurement)	Advisory	29/04/16
Energy and Natural Resources Group chaired by HFS.	Local engagement with stakeholders to secure optimum value for money option	29/04/16

QuestionName	DisplayOrder	TopicName	Response	AdditionalInformation	EvidenceLocation	RiskLikeliness	RiskSeverity	Risk	Calculation	RiskComments
EAMS 42.01 - Have the domestic hot & cold water systems been installed according to the principles set out in SHTM 04-01?	1	Water 2014	4	Systems: Systems in place throughout the site, documentation held	The process of regularly reviewing and updating the site Risk Assessments and Written Scheme has assisted in identifying any non-compliant installation issues. This process is ongoing. RAs and WS documents are stored on the QEUH Shared Drive in the Water Quality folder.	3	2	6	3x2	The degree of scrutiny and governance applied to this topic has led to an increased level of control of the water system, therefore reducing the risk rating
Are domestic hot & cold water systens being maintained according to the principles set out in SHTM 04-01?	2	Water 2014	4	The principles of SHTM 04-01 have been adhered to in forming the updated Written Scheme which gives guidance on the specific maintenance activities required to maintain the water system.		2	3	6	2x3	The guidance given in the written scheme is clear and concise.
Are domestic hot & cold water systems operational and maintenance guidance reviewed after a change in environmental use, a system alteration or a change to guidance to ensure they remain appropriate?	3	Water 2014	4	New written scheme for the QEUH campus contains a requirement for the document to be reviewed on a 6 monthly basis which will take guidance, change of use and alterations into account.	\\sgd-fs-vs\S-Estates$\Water Quality\Written Schemes	2	4	8	2x4	Agree that change in use / alterations going unnoticed is unlikely but believe consequence sits in the major category - especially with new control measures in the written scheme in place.
Are records, logbooks etc., regarding commissioning data, seasonal commissioning, management routines and PPM retained and available for inspection?	4	Water 2014	4	The updated written scheme defines which tasks should be carried out and on which frequencies. This also clearly defines the process of recording and storing the maintenance information on an ongoing basis.	https://scartscartprodblob.blob.core.windows.net/evidence/2018/212/7df b1238-cbe2-4e36-b3bc-4eafe96f5f78.pdf	3	1	3	3x1	New defined process in place.
Have all individuals with roles and responsibilities, as defined in the guidance, been appointed with acceptance confirmed in writing?	5	Water 2014	4	Currently there is 1 x DRP 1 x Lead AP with the support of 4 x additional APs and 9 x CPs all trained and appointed in writing to serve at the QEUH site.	Training and Appointments folder within the Estates Shared Drive. Also held on Smartsheet by the Compliance Team.	1	2	2	1x2	I would consider the answer to this question is fully compliant and risk mitigated.
Are management roles and responsibilities reviewed periodically (i.e. not exceeding 3-year intervals) and recorded?	6	Water 2014	4	Roles and responsibilities are defined in the updated written scheme which will be reviewed on at least an annual basis.	https://scartscartprodblob.blob.core.windows.net/evidence/2018/212/222 5b823-0817-4155-b58f-eaecade0c9d0.pdf	1	1	1	1x1	Process is in place and impact of this is low.
SW - Are appropriate governance arrangements in place to ensure that the Estates Department is represented on the appropriate committees?	7	Water 2014	4		Water Group and Infection control	1	1	1	1x1	
Are adequate numbers of competent staff available to fulfil the requirements of SHTM 04-01 and cover for eventualities (sickness, holidays?)	8	Water 2014	4	Improvements have been made with regards to CP training. Number of trained CPs currently at 9. Contract labour is also being utilised to assist with compliance with regards to water quality & PPMs.	All water systems maintenance records are stored within the Main Estates Office under the control of the Lead AP.	1	3	3	1x3	
Do all water systems have a current applicable Legionella Risk Assessment to BS 8580?	9	Water 2014	4	Risk: Risk assessments in place, held on central drive	Paper records are held within the Estates Main Office	1	4	4	1x4	
Was the person carrying out the Risk Assessment competent (BS 8580) or accredited to do so?	10	Water 2014	4	RAs carried out by DMA Water	RAs held in Main Estates Office	1	4	4	1x4	
Were the significant findings of the Risk Assessment identified, prioritised and recorded?	11	Water 2014	4	Action Plan: Action plan in place	Action plan for each building/location held within the risk assessment documentation	1	4	4	1x4	
Is there a real time Action Plan to address the significant findings of the Risk Assessment?	12	Water 2014	4	Action Plan: Action plan in place	Action plan for each building/location held in risk assessment documentation	1	4	4	1x4	
Are all relevant staff aware of the Action Plan?	13	Water 2014	4	AP, DRP & RP are all aware. AP share applicable sections of the action with the maintenance technicians who will carry out the work.	RAs on shared drive.	1	4	4	1x4	
EAMS 42.99 - Have the high risks in the Action Plan been mitigated/implemented?	14	Water 2014	4	Action plan in place and tracked on Smartsheet. All high risk items from previous RA action plans complete.	Closed actions tracked on Smartsheet.	1	5	5	1x5	
EAMS 42.99 - Have the medium risks in the Action Plan been mitigated/implemented?	15	Water 2014	4	Action Plan: Medium risks from A&C plan closed.	Closed actions tracked on Smartsheet	1	4	4	1x4	
EAMS 42.99 - Have the low risks in the Action Plan been mitigated/implemented?	16	Water 2014	4	All actions complete	Closed actions tracked on Smartsheet	1	3	3	1x3	
SW - Is there a current and up-to-date Water Quality Policy?	17	Water 2014	4	Updated Policy and Procedures Policy has been approved and distributed Friday 8th December 2017		1	4	4	1x4	
Have all individuals with Roles & Responsibilities for specific Written Schemes been appointed in writing?	18	Water 2014	4	Those responsible for the written schemes are the AP, RP and DRP who have all been appointed in writing.	Records can be found in individuals personal files and copies will also be included in the master written scheme.	1	4	4	1x4	
Have all individuals with Roles & Responsibilities received appropriate up-to-date training?	19	Water 2014	4	Now have 3 trained and signed off APs. One AP trained and to be signed-off. CP training has been carried out. Some additional staff may be considered for CP training (fitters / MAs).		1	4	4	1x4	
Are contact details for these people readily available?	20	Water 2014	4	Contact details known to those in the estates team. Need to consider what level of visibility of contact details are required outwith Estates / Facilities.	Within written scheme on SGH shared drive/Water Quality/Written Schemes	1	2	2	1x2	
Is there regular annual review of Written Schemes to take cognisance of the risks associated with new technologies?	21	Water 2014	4	New written scheme has been developed for the site which includes the procedure for reviewing the content and also specifies what needs to be reviewed and considered.	SGH shared drive/Water Quality/Written Schemes	1	4	4	1x4	
Is there a Written Scheme (Operational Procedures) in use for Control of Legionella in each Domestic Water System?	22	Water 2014	4	Yes. Written Scheme is regularly reviewed.	SGH Estates Shared Drive within the "Water Quality" folder.	1	4	4	1x4	
EAMS 42.99 - Are there accurate 'As-Fitted' drawings of all the Domestic Water Systems?	23	Water 2014	3	26/03/20 Additional Drawings require to be updated when additional resources are available.	All available drawings and schematics are stored on the QEUH Shared Drive CAD Drawings Folder System.	3	2	6	3x2	Previous risk rating of 20 was considered too high for the impact of unavailable or inaccurate drawings.
Does the Written Scheme (Operational Procedures) contain clear and specific instructions for the safe management of each domestic water system?	24	Water 2014	4	Yes, this is specified in the new site written scheme.	SGH shared drive/Water Quality/Written Schemes	1	4	4	1x4	
EAMS 42.19 - Have you ensured that all components within the Domestic Water Services systems are Water Byelaws compliant and listed in the Water Fittings and Material Directory as published by the Water Research and Advisory Service (WRAS)? (Ref SHTM 04-01Part A, P14 Para 1.10)	25	Water 2014	3	23/03/20 Reveiwed and still do not have a accurate component level asset register which would be required to fully comply. Where reasonably practicable we have ensured that materials and fittings installed on the system are WRAS approved. Control measures are in place through a permit to work system and continual water quality analysis to ensure that any future alterations to the system are done in a controlled manner and risk is mitigated.		3	3	9	3x3	The previous risk rating of, Almost Certain x Major is thought to be too high. It is possible that Non-WRAS fittings could have been installed inadvertently in the water systems hisorically but would rate the severity of that as being moderate as opposed to Major. Control measures are in place to monitor water quality.
EAMS 42.23 - Are thermostatic mixing valves sited as close as possible to the point of use?	26	Water 2014	4	This is the case for A&C as TMTs are used throughout the installation ensuring that water is mixed at point of use. Thermostatic showers also aid compliance with this requirement. There are no reports of non-compliance from other buildings on campus.	TMV/TMT service records from DMA.	1	5	5	1x5	
EAMS 42.99 - Are low use cold water service outlets installed prior to high use outlets?	27	Water 2014	-1	This has been marked N/A since there is no way to be certain, even if installed this way, that clinical / user activity would match design.		0	0	0	0x0	
EAMS 42.02 - Has cold water storage been optimised i.e. holds enough for a day's operational use only?	28	Water 2014	4	The water tanks within the retained estate are configured to store approx 12 hours of supply. The adult and childrens tanks are configured to store approx 24hrs supply at the moment. Drop tests to be carried out on an annual basis to confirm the position.		1	2	2	1x2	Adequate turnover of the water storage. Ultrasonics in tanks in A&C allows ongoing monitoring or adjustments of levels e.g. during COVID in 2021.
EAMS 42.02 - Is the cold water system pipework insulated where possible?	29	Water 2014	4			1	4	4	1x4	
EAMS 42.02 - Is the cold water system pipework kept away from heat sources?	30	Water 2014	4			1	4	4	1x4	
EAMS 42.02 - Does the cold water storage tank comply with the Water Bye-Laws?	31	Water 2014	4	A number of modifications to tanks have been carried out to make compliant. Main tanks in A&C have been identified as non compliant and forms part of the legal claim and this is being progressed by that team. 05/06/23 SBAR created for rectification team.		1	4	4	1x4	
EAMS 42.02 - Is the cold water system designed and constructed to ensure that it is maintained at 20C or below?	32	Water 2014	4	All pipework and storage tanks are adequately insulated to avoid heat gain. Incoming cold water temperatures are dependent on the ambient supply from Scottish Water. This is continually monitored on BMS and will generate alarms if above 20C. Enhanced monitoring is carried out during summer months.	BMS trend graphs	1	4	4	1x4	
EAMS 42.04 - Is the water temp pick up less than 2C from tank to furthest away sentinel tap?	33	Water 2014	4	The incoming water temperatures and system temperatures are monitored via the BMS system. These indicate no more than a 2 degC increase from source.		1	4	4	1x4	

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EAMS 42.05 - the hot water generator storage at no less than 60C?	34	Water 2014	4	All hot water storage is set for temperatures of 60 deg C or above.	Can be viewed on the BMS set points. This is recorded regularly and can be displayed as a trend graph.	1	4	4	1x4	
EAMS 42.05 - Does the normal daily storage capacity of domestic hot water meet the requirements of the minimum fluctuations in temperature of domestic hot water (i.e. a max of 20 mins temperature recovery time)?	35	Water 2014	4	Only issue is with the energy centre control strategy which is currently being reviewed. A file has been attached which demonstrates that the calorifiers are consistently maintained at temperature throughout the high demand periods.	https://scartscartprodblob.blob.core.windows.net/evidence/2019/205/c63fbe18-db9a-4f04-8906-7b73da2b137c.pptx	1	4	4	1x4	
EAMS 42.08 - Is a minimum temp of no less than 55C maintained at furthest away domestic hot water point?	36	Water 2014	4	This is currently being monitored manually on a daily basis until such time as critical alarms for return temperatures are in place and being monitored by the Helpdesk. If at any stage a temperature issue is identified, the person reviewing the temperatures takes action or escalates the issue.		1	4	4	1x4	
EAMS 42.07 - While in use, is the return temperature of the domestic hot water maintained at no less than 50C at the hot water generator?	37	Water 2014	4	This is currently being monitored manually on a daily basis until such time as critical alarms for return temperatures are in place and being monitored by the Helpdesk. If at any stage a temperature issue is identified, the person reviewing the temperatures takes action or escalates the issue.		1	4	4	1x4	
EAMS 42.05 - Are the domestic water distribution pipes insulated?	38	Water 2014	4			1	4	4	1x4	
EAMS 42.99 - Does the Domestic Hot Water generating plant comply with the relevant paragraphs of Section 9 of SHTM 04-01 Part A, (9.9 et seq) ?	39	Water 2014	4	Having reviewed SHTM 04-01 Part A, this is specifically looking at heat generating plant so is interpreted as sections 9.9 to section 9.15 inclusive. THESE SECTIONS TO BE REVIEWED BY AP AND ANSWER INCLUDED HERE.	As per sections 9.9 to 9.15 the Heat generating equipment primary side is MTHW which flows through individual plate heat exchangers which heat up the domestic cold water (secondary side) within a vertical Calorifier, access hatches and safety features are all compliant as per SHTM's	1	4	4	1x4	
If the water supplied to your site is not from mains supply (i.e. it is from a private supply), has the water been pre-treated to make sure it is of the same quality as specified in the European Drinking Water Directive?	40	Water 2014	-1			0	0	0	0x0	
EAMS 42.99 - Are the entire contents of the heat generating equipment, including the base, heated to 60C for an hour each day, for example by using a de-stratification pump?	41	Water 2014	4			1	4	4	1x4	
EAMS 42.10 - Are all outlets that are no longer required cut back as far as the main circulating pipe-run with branch tee removed and straight coupling substituted?	42	Water 2014	4	This has been addressed.		1	4	4	1x4	
Are there arrangements in place to incorporate standby equipment, for example heat generating equipment and pumps into routine use?	43	Water 2014	4	In cases where we have a duty and standby pump plumbed into the system, a control strategy is in place to ensure roation of use.		1	4	4	1x4	
If infrequently used outlets have not been removed, are there formal arrangements in place to flush them through on at least a twice-a-week flushing cycle?	44	Water 2014	4	This is carried out by domestic cleaning staff except where a little-used-outlet is identified to estates as requiring flushing by estates.		1	4	4	1x4	
Is there a log kept of the frequency of flushing of infrequently used outlets?	45	Water 2014	4	Flushing which is the responsibility of estates is recorded. The task for this is controlled through FM First and the log is kept on a standard template in the site log books. A quarterly memo is sent out via email to all SCN's to remind them of the responsibility to record flushing of any little used outlets or report them to Estates for removal.		1	4	4	1x4	
Where thermostatic mixing valves are fitted, are they included in the maintenance schedule?	46	Water 2014	4	This is being carried out by on-site contractor DMA		1	4	4	1x4	
Where thermostatic mixing valves are fitted, are they maintained as per the schedule and recorded?	47	Water 2014	4	26/03/2020 Minor service has now been completed by contractor DMA Canyon, major maintenance planned for july / august 2020	All records will be held in SCART Water 22 folder on Estates shared drive.	1	4	4	1x4	
Is there a water treatment regime in place, including thermal disinfection?	48	Water 2014	4	CL02 in place and fully monitered by Scotmas.	Currently held by Scotmas and will be downloaded to SCART water 22 folder.	1	4	4	1x4	
Are Risk Assessments carried out prior to implementing any form of water disinfection regime ? (Ref SHTM 04-01 Part A, P 83 Section 15 Para 15.1)	49	Water 2014	4	Risk assessments are carried out by the sole contractor carrying out such works (DMA Water). These are submitted for approval prior to the works taking place.	https://scartscartprodblob.blob.core.windows.net/evidence/2018/212/0127d038-f495-4d1d-a2fe-2b8ced25194a.pdf	2	3	6	2x3	RAs carried out
Do you control, monitor & record any water disinfection (and any associated treatment) regime(s) you have in place?	50	Water 2014	4	Scotmas carry out monthly, 6 monthly and annual checks of CL02 systems. Tank and Calorifier records also reatained by DMA Canyon	Planned to be migrated over to SCART Water 22 file.	1	4	4	1x4	
Is the temperature of sentinel hot and cold outlets checked on a monthly basis and recorded? (For cold water the temp should be <20C within 2 mins; for hot water > 50C within I min)	51	Water 2014	4	This is being done by direct labour in the A&C buildings and carried out by contractor DMA in the retained estate buildings.	All records are kept in the Main Estates Office under the control of the Lead AP.	1	4	4	1x4	
Is the domestic hot water temperature of the outlet and return pipes of the heat generating equipment checked on a monthly basis and recorded (60C hot outlet, 50C minimum return)?	52	Water 2014	4		Manual checks are made on some heat generating equipment and other are recorded on trends from the BMS	1	4	4	1x4	
Is the temperature of the incoming domestic cold water supply checked on a six-monthly summer / winter basis and recorded?	53	Water 2014	4	Incoming water temperatures are checked more frquently than this via the BMS.		1	4	4	1x4	
Is the temperature of at least 20% of all domestic hot and cold water outlets checked on an annual basis and recorded over a 5 year programme?	54	Water 2014	4	Systems: Systems in place throughout the site, documentation held. Sentinels are checked monthly through out the campus between NHS and DMA Canyon. Programme in place for minor service of TMT's, Shower valves and Contour TMT taps (which includes temperature checks as per SHTM's).	All records are kept in the Main Estates Office under the control of the Lead AP. Temperatures of taps are taken as part of ongoing sampling, Sentinel outlet flushing and TMT maintenance.	1	4	4	1x4	
Are there procedures in place to identify circumstances when either general microbiological monitoring or sampling for legionella would be appropriate (i.e. in the Written Scheme (Operational Procedures))?	55	Water 2014	4	This is covered in detail in the written scheme for the QEUH campus.	\\sgd-fs-vs\S-Estates$\Water Quality\Written Schemes	1	4	4	1x4	
If there are procedures in place, do these identify where samples should be taken, and the frequency and actions required?	56	Water 2014	4	The written scheme dictates the cases in which sampling is required whether this is an identified engineering risk or at request of infection control. Location is determined by engineering risk identified or IC.	\\sgd-fs-vs\S-Estates$\Water Quality\Written Schemes	1	4	4	1x4	
Have you ensured that there is an Action Plan in place to address any disinfection requirements such as system alterations, outbreak, high Legionella counts, recorded temperatures outwith parameters or new works, etc? (Ref SHTM 04-01Part A,)	57	Water 2014	4	Detailed in the written scheme. SHTM 04-01 part B dictates the specific requirements of disinfection and leechate flushing. This is referred to from the written scheme.	\\sgd-fs-vs\S-Estates$\Water Quality\Written Schemes	1	4	4	1x4	
Do you have monitoring procedures in place, authenticating and recording TMV types and discharge temperatures from all appliances? (Ref SHTM 04-01 Part A, P11&12)	58	Water 2014	4	This is currently being carried out by on-site specialist contractor DMA.	This work has been started by DMA Canyon (May / June 2019). Minor Service. All records are kept in the Main Estates Office under the control of the Lead AP.	1	4	4	1x4	
If a TMV is fitted, is the temperature of the water supply the TMV checked on a six-monthly basis? Where duplication refer to previous question	59	Water 2014	4	This is currently being carried out by in-house specialist contarctor DMA	This work has been started by DMA Canyon (May / June 2019). Minor Service. All records are kept in the Main Estates Office under the control of the Lead AP.	1	4	4	1x4	
EAMS 42.21 - Have you ensured that all warning signs for Caution Very Hot Water are legible, highly visible and securely attached, close by to relevant outlet points? (Ref SHTM 04-01 Part A, p12)	60	Water 2014	4	These are installed as required within the retained estate.		1	4	4	1x4	
EAMS 42.23 - If there is a risk of scalding (for example anywhere the young, elderly, disabled or staff may use the outlets), are TMVs fitted?	61	Water 2014	4	Yes, this is the case.		1	5	5	1x5	
EAMS 42.24 - Has the NHS Board made arrangements for low surface temperature emitters* to be located in areas occupied by vulnerable patients and staff? * NB This would include ceiling-mounted emitters	62	Water 2014	4	This is captured at design stage of projects.		1	4	4	1x4	
Do you have a regime to deal with emergency failure of incoming water supply? (Ref SHTM 04-01 Part A, p18, Para 2.3)	63	Water 2014	4	Arrangements are in place with Anglian water to ensure emergency response		1	4	4	1x4	

If there is a requirement for a Contaminated Land Schedule for this site, do you have one? (ref SHTM 04-01 Part A, P19 Para 2.7)	64	Water 2014	4	Section 5 of the attachment covers previous land uses and sampling carried out for the permit application. If there was any significant contamination this would have been flagged up and remediated. Anything else that was potentially suspect, we would still be monitoring as part of the permit conditions. So in summary no, there is no need for a Contaminated Land Register. This summarised in Section 7 of the evidence attachment.	https://scartscartprodblob.blob.core.windows.net/evidence/2018/325/54809687-bea7-4bc3-8378-795c8f6cce25.docm	1	4	4	1x4	
Has the NHS Board established a Water Safety Plan in accordance with Guidance for neonatal units (NNUs) adult & paediatric intensive care units (ICUs) to minimise the risk of Pseudomonas aeruginosa infection from water?	65	Water 2014	4	Policy: Policy / procedure in place, held on central drive	https://scartscartprodblob.blob.core.windows.net/evidence/2018/212/78c704b9-4ebc-4314-acbe-343beccd60ea.pdf	3	2	6	3x2	Policy guidance is followed
SW - Has the NHS Board established a Water Safety Group with appropriate representation from all stakeholders to ensure that a coordinated approach is pursued between IPCT, clinical staff and Estates/Facilities on all water issues?	66	Water 2014	4	Systems: Systems in place throughout the site, documentation held	All records kept within the GGH Estates Shared Drive	1	2	2	1x2	Water Safety Group Established and all Meetings minuted
SW - Have the roles and responsibilities of the members of the Water Safety Group been clearly set out and recorded?	67	Water 2014	4	Systems: Systems in place throughout the site, documentation held		1	5	5	1x5	
SW / EAMS 42.99 - Has the Water Safety Group taken steps to ensure that splashes from water outlets are minimised?	68	Water 2014	4			1	5	5	1x5	
SW - Does the Water Safety Group have systems in place to ensure that flushing of all taps is being performed for 1 minute daily?	69	Water 2014	4			1	5	5	1x5	
SW - Has the Water Safety Group identified, in conjunction with the relevant Senior Charge Nurse, those wash hand basins that are infrequently used (i.e. once per day)?	70	Water 2014	4			1	5	5	1x5	
SW - Has the Water Safety Group set up a documented flushing regime for these infrequently used wash hand basins?	71	Water 2014	4			1	5	5	1x5	
SW / EAMS 42.99 - Has the Water Safety Group made arrangements for all redundant branches from circulating mains to be removed including plugged tee-pieces?	72	Water 2014	4			1	5	5	1x5	
SW / EAMS 42.99 - Has the Water Safety Group made arrangements to locate TMVs as close as possible to outlets?	73	Water 2014	4			1	5	5	1x5	
SW - Has the Water Safety Group made arrangements for all new taps to be capable of having point-of-use filters retrofitted?	74	Water 2014	4	All the decisions on new taps would go through the Water Technical Group. Were this ever dispanded, this would be covered by Sector and Board Water Safety Groups to ensure this was the case. This is the case for new taps being installed in adult's and children's.		1	4	4	1x4	

UK Standards for Microbiology Investigations

Investigation of superficial mouth samples

National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

Issued by the Standards Unit, UKHSA

Bacteriology | B 4 | Issue no: 7.2 | Issue date: 23.08.22 | Page: 1 of 20

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UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of the UK Health Security Agency (UKHSA) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

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UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 6

SCOPE OF DOCUMENT 8

INTRODUCTION 8

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 11

2 SPECIMEN COLLECTION 11

3 SPECIMEN TRANSPORT AND STORAGE 12

4 SPECIMEN PROCESSING/PROCEDURE 12

5 REPORTING PROCEDURE 15

6 NOTIFICATION TO UKHSA, OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 16

APPENDIX: INVESTIGATION OF SUPERFICIAL MOUTH SAMPLES 17

REFERENCES 18

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	12/23.08.22
Issue no. discarded.	7.1
Insert issue no.	7.2
Section(s) involved	**Amendment**
Introduction	Correction made to the link on page 8 from UK SMI B17 Investigation of tissues and biopsies to UK SMI B 14: investigation of pus and exudates

Amendment no/date.	11/02.12.15
Issue no. discarded.	7
Insert issue no.	7.1
Section(s) involved	**Amendment**
4.5.3 Culture media, conditions and organisms.	Error in atmosphere column corrected.

Amendment no/date.	10/20.10.15
Issue no. discarded.	6.3
Insert issue no.	7
Section(s) involved	**Amendment**
Whole document.	Document restructured, rewritten and expanded to meet the requirements of the new scope. Hyperlinks updated to gov.uk.
Title of the document.	Changed to capture more sample types.
Page 2	Updated logos added
Types of specimen	Saliva and oral rinses added in

Culture	Amended to include new sample types
References	Reviewed and updated

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with UKHSA, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

UKHA is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to UKHSA Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, UKHSA and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

UK Health Security Agency. (2015). Investigation of Superficial Mouth Samples. UK Standards for Microbiology Investigations. B 4 Issue 7. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Mouth swab, saliva and oral rinse

This SMI describes the processing, and bacteriological and mycological investigation of superficial mouth samples. Predominately mouth swabs but saliva and oral rinses are also covered. Infections of salivary glands (parotid, submandibular and sub-lingual) include bacterial and viral infections and are not covered in this SMI.

This SMI should be used in conjunction with other SMIs.

Introduction

Infections of the oral mucosa usually present as acute conditions. Usually these arise from the colonising oral flora but can also result from a flare-up of a chronic low-grade infection.

Oral mucosal infections are typically associated with biofilms formed on the inanimate surfaces present in the oral cavity such as the teeth and dentures.

Infections of the gingiva (gingivitis, including acute ulcerative gingivitis) and periodontal tissues (periodontitis) are the most common forms of oral infection and processing specimens from these infections are covered in UK SMI B 14: investigation of pus and exudates.

Oral mucositis

Oral mucositis is a painful complication of chemotherapy or head and neck radiotherapy, caused by direct cytotoxicity of the treatment regime. Super-infection usually with yeasts and oral bacteria can exacerbate the problem and microbiological examination can help to guide symptomatic treatment.

Erythematous and pseudomembranous candidosis[1,2]

Erythematous and pseudomembranous candidosis are the most frequent clinical presentations of oral fungal infection. The infections may involve the mucosal surfaces of the cheeks, tongue (dorsal and ventral surfaces) and both hard and soft palates. The most common cause is *Candida albicans*. *Candida* species other than *C. albicans* such as *Candida glabrata* may also be isolated, either alone or in combination with *C. albicans*. This is especially common in the medically compromised or those with a history of prolonged antifungal therapy[3,4]. Atrophic candidosis (denture stomatitis) may occur in the palatal mucosa below the fitting surface of dentures, especially when patients sleep with their dentures in place and/or have xerostomia. *Candida* species other than *C. albicans* are important to identify, since they may demonstrate reduced susceptibility and clinical resistance to the first line anti-fungal agents and may be responsible for refractory or recurrent infections. Rarely, moulds may colonise and infect sinuses and result in palatal erosion. Specimens in the form of an oral rinse (known volume of sterile saline) are used to quantitatively determine colonisation or infection[5].

Angular cheilitis and peri-oral infections

Angular cheilitis and peri-oral infections are common infections affecting the angles of the mouth and lips, usually caused by an intra-oral reservoir of infection, typically biofilms associated with denture stomatitis. Infection may be due to *S. aureus*, *Candida* species and/or Group A streptococci. It is common for dentate patients with angular cheilitis to have infection with both *S. aureus* and *C. albicans* in the labial commissure region. Swabs should be taken from the lesions themselves. Swabs should also be collected from relevant intra-oral sites for example, denture-fitting surface and the anterior nares to identify sites of colonisation to be treated with eradication therapy, to reduce relapse rates.

Staphylococcal mucositis[6]

Patients who are severely medically compromised and have reduced salivary flow, together with parenteral feeding, may develop staphylococcal mucositis caused by *S. aureus.* Enterobacteria may also play a role in severe cases. The erythematous changes in the oral mucosa may be indistinguishable clinically from candidosis, requiring the need for microbiological investigation. Results should be interpreted in a clinical context since asymptomatic carriage of *S. aureus* or Enterobacteria may occur. Strict regular oral hygiene measures are usually sufficient to resolve clinical symptoms. Systemic antibiotics are not usually required although may play an important role in the management of severe oral mucositis in some patient groups such as the terminally ill.

Oral ulceration

There are many non-infective causes of oral ulceration such as traumatic ulcers, recurrent aphthous ulcers, inflammatory conditions and malignant lesions. Infective causes of oral ulceration are commonly viral in origin (for example, Herpes simplex). Uncommon bacterial causes of ulceration are syphilis and tuberculosis whilst other rare causes of oral ulceration include fungal infections such as histoplasmosis.

Abscess and deep seated infections

Abscess and deep seated infections (dental abscesses, and salivary gland abscesses) are dealt with in B 14 - Investigation of abscesses and deep seated wound infections.

Osteomyelitis

Osteomyelitis, including bacterial, mycobacterial and fungal osteomyelitis are dealt with in B 42 - Investigation of bone and soft tissue associated with osteomyelitis.

Vincent's angina

Borrelia vincentii and *Fusobacterium* species are associated with the infection known as Vincent's angina. It is characterised by ulceration of the pharynx or gums and occurs in adults with poor mouth hygiene or serious systemic disease[7]. See B 9 – Investigation of throat related specimens.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[8,9]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

1 Safety considerations[8-24]

1.1 Specimen collection, transport and storage[8-13]

Use aseptic technique.

Collect saliva and oral rinse specimens into appropriate CE marked leak proof containers and transport specimens in sealed plastic bags.

Use tubes with transport medium for transporting swabs and transport in sealed plastic bags[25].

Transport each swab in transport medium in a CE marked container in a sealed plastic bag.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[8-24]

All Hazard group 2 organisms must be confirmed at containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[16].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

If there is histoplasma (and/or other relevant dimorphic pathogens causing oral ulceration) risk then containment level 3 is required using an appropriate cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Mouth swab, saliva and oral rinse

2.2 Optimal time and method of collection[26]

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[26].

To assure that the preconditions of the sampling for oral infections are comparable it is advised that patients should not:

1. eat or drink within 2 hours
2. brush their teeth within 2 hours
3. use any mouth rinse of disinfectant within 2 hours prior to sampling

If possible samples should be taken in the morning under fasting conditions.

Unless otherwise indicated collect each swab for bacterial and/or fungal culture and place in appropriate transport medium[25,27-30].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Sample any lesions or inflamed areas using cotton tipped swabs. Samples of denture fitting surfaces should also be swabbed as these are more sensitive sites than the palatal mucosa to recover *Candida* species. The use of a tongue depressor or spatula may be helpful. Oral rinses can be useful to follow up level of colonisation. These are collected by rinsing with 10mL of sterile saline for one minute.

2.3 Adequate quantity and appropriate number of specimens[26]

Numbers and frequency of specimens collected depend on the clinical condition of patient.

3 Specimen transport and storage[8,9]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[26].

Collect mucosal swabs in transport medium which should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature.

Oral rinses should be transported in a CE marked leak proof containers and placed in sealed plastic bags and processed as soon as possible.

4 Specimen processing/procedure[8,9]

4.1 Test selection

Most mouth samples are swabs unless the patient is immunocompromised or has other clinical indications.

Saliva samples may be collected for microbiological investigation and for other types of assessment. Increasingly saliva is being used as a sample for new diagnostic techniques, but also for assessing xerostomia and risk of dental caries. Care is needed to avoid contamination of these specimens and cross infection from these specimens. Sometimes culture is done with an exact volume of saliva in order to assess the count of a particular organism (for example *S. mutans* or lactobacilli per mL of the original saliva sample.

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

For oral rinses (saliva/mouth washings) centrifuge at 3200 rpm for 10 minutes.

Decant supernatant into disinfectant and re suspend the deposit in 1mL PBS.

This is now the neat sample.

Inoculate 50µL onto a sabouraud agar plate using a hockey stick to spread out for single colonies and a columbia agar plate.

For comparison it is sometimes useful to dilute neat sample 1:100 (0.1mL + 9.9mL PBS). Inoculate 50µL onto a Columbia Blood Agar and use a hockey stick to spread out. A MacConkey/Cystine lactose electrolyte deficient agar (CLED) plate may also be useful.

4.4 Microscopy

Direct microscopic examination with Calcofluor staining may be helpful if histoplasma or mould infection is suspected.

4.5 Culture and investigation

Inoculate each agar plate using a sterile loop or a loopful of liquid (Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	**Specimen**	**Standard media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Oral candidosis Fungal infection	Mouth swab, Saliva and oral rinse	Sabouraud agar	35-37	Air	40-48hr*	Daily	*C. albicans,* Non-albicans yeasts
Oral erythema Denture stomatitis Angular cheilitis Mouth ulcer		Blood agar	35-37	CO_2 5-10%	16-24hr	daily	Group A, strep, *S. aureus,* Coliforms
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Oral mucositis Immunocompromised patients	Mouth swab, Saliva and oral rinse	MacConkey/CLED agar Chromogenic agar	35-37 35-37	Air Air	16-24hr 16-24hr	daily daily	*Coliforms* and non-fermentative gram negatives Candida species
*If Histoplasmosis is suspected the length of incubation should be extended and carried out in Containment Level 3. If an unusual fungal infection is suspected a second Sabouraud plate should be set up at 30°C and incubation time extended.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Yeasts	Yeasts level Patients showing treatment failure require a full identification.
Staphylococcus aureus	species level
Lancefield group A streptococcus	species level
Enterobacteriaceae species	"coliform" level if dominant growth

Organisms may be further identified if this is clinically or epidemiologically indicated.

Immunocompromised Patients

Candida species	species level
Aspergillus species and other moulds	genus level
Staphylococcus aureus	species level
Lancefield group A streptococcus	species level
Coliforms	Coliforms level or if clinically indicated species level
Pseudomonas	Pseudomonas level if dominant growth or if clinically indicated species level
Acinetobacter	*Acinetobacter* level if dominant growth or if clinically indicated species level
Stenotrophomonas	Stenotrophomonas level if dominant growth or if clinically indicated species level

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

C. albicans is not routinely tested unless associated with recurrent infection, requested by clinician or the patient's history indicates significant immunosuppression.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Report for fungi if applicable.

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

Report clinically significant organisms isolated **or**

Report other growth, eg: “Mixed upper respiratory tract flora” **or**

Report absence of growth or

Report presence or absence of specific named pathogens

Report quantitative growth if applicable. For rinses report as[5]:

Heavy growth: >10^4cfu/mL

Moderate growth = 10^2-10^3cfu/mL

Light growth = <10^2cfu/mL

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to UKHSA[31,32], or equivalent in the devolved administrations[33-36]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify UK Health Security Agency (UKHSA) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local UKHSA Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

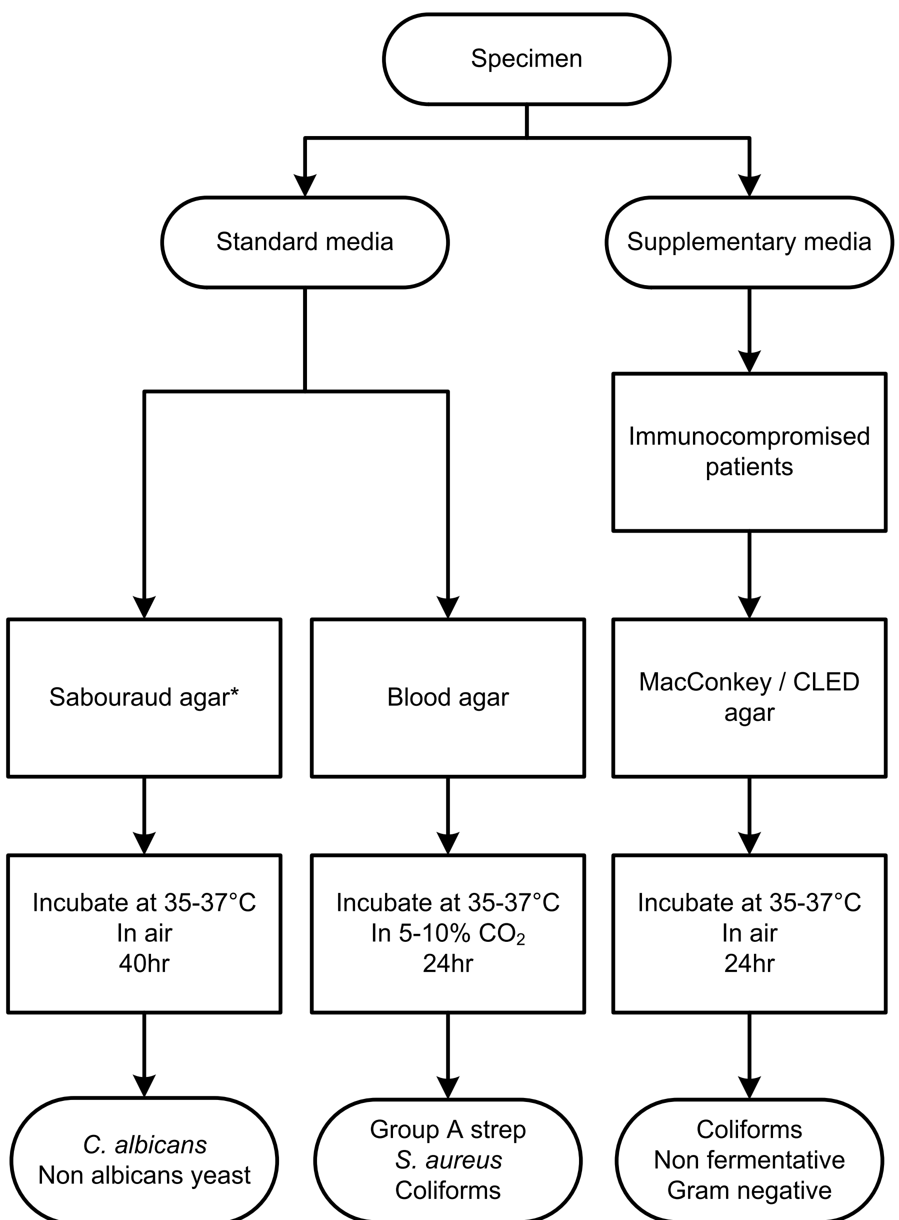
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to UKHSA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to UKHSA and many UKHSA Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[33,34], Wales[35] and Northern Ireland[36].

Appendix: Investigation of superficial mouth samples



*If histoplasmosis is suspected the length of incubation should be extended and carried out in Category 3 conditions.

If an unusual fungal infection is suspected a second Sabouraud plate should be set up at 30°C and incubation time extended.

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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of users' comments received by

Working group for microbiology standards in clinical bacteriology

B 4 Investigation of superficial mouth samples

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation: 25/02/2013 – 20/05/2013

Version of document consulted on: B 4dc+

Proposal for changes

Comment number	1		
Date received	05/05/2013	**Lab name**	St Georges Healthcare NHS Trust
Section	General, page 16		
Comment			
General comments: A lot of laboratories reported that they do not examine mouth swabs unless there are details of recurrent infection ie candidiasis. Mention that pus is received from the parotid gland then this should be processed a pus specimen and refer to the SMI. Page 16 - Vincent's organisms should be included in the flowchart.			
Recommended action	**ACCEPT** These issues were addressed when the document was rewritten.		

Comment number	2		
Date received	14/05/2013	**Lab name**	Glasgow Royal Infirmary
Section	All		
Comment			
On behalf of the Association of Clinical Oral Microbiologists we have multiple comments and revised flowchart for this SMI and have e-mailed the revised document.			
Recommended action	**ACCEPT** These issues were addressed when the document was rewritten.		

2nd Consultation: 23/12/2013 – 20/01/2014

Version of document consulted on: B 4dj+

Proposal for changes

Comment number	1		
Date received	23/12/2013	**Lab name**	University of British Columbia

Section	All
Comment	
With respect, in my opinion this is a document of obsolete microbiology. I think that the standard should consist solely of the following: The collection of Superficial Mouth Samples for microbial analysis is discouraged.	
Evidence	
Mouth flora is a dense mix of aerobic and anaerobic bacterial and fungal and viral flora. Some is resident, much is transient. Some is commonly thought of as pathogenic much is thought of as benign. The impact of the collective flora taken in is impossible to interpret. The interpretation of any single organism regardless of quantity is impossible. Treatment of superficial mouth sores with antibiotics or antifungals is questionable more effective that oral washes. Adding in antibiotics may contribute to altered flora illness (such as *C. diff*) In some instances use of anti-candida lozenges may appear to be beneficial even if thrush is not materially present.	
Financial barriers	
No.	
Health benefits	
Yes. See section 6.	
Recommended action	**NONE** Although we agree with essence of the comment in certain circumstances and certain patient groups it is still a useful method.

Comment number	2		
Date received	24/12/2013	**Lab name**	past clinical microbiology laboratory Careggi Hospital Florence
Section	4.5.1		
Comment			
Pag. 12 tab. 4.5.1 Oral candidosis and fungal infections are not clinical conditions but microbiological diagnosis.			
Financial barriers			
No.			
Recommended action	**NONE** The table states clinical details/conditions and is standard in all UK SMIs.		

Comment number	3		
Date received	14/01/2014	Lab name	University Giessen (JLU), Dept. of Periodontology, Germany
Section	Various		
Comment			
To assure that the preconditions of the sampling are comparable for all patients it seems reasonable that patients before sampling: 1. didn't eat or drink within 2 hours 2. did not brush their teeth within 2 hours 3. did not use any mouth rinse of disinfectant within 2 hours prior to sampling If possible samples should be taken in the morning under fasting conditions			
Recommended action	ACCEPT This text has been added to the document.		

Comment number	4		
Date received	19/01/2014	Lab name	Glasgow Royal Infirmary
Section	Section 4		
Comment			
ORAL RINSE An example of a method used to process oral rinses; Centrifuge at 3200 rpm for 10 minutes. Decant supernatant into disinfectant and resuspend the deposit in 1 ml PBS. This is now the neat sample 1. Inoculate 50ul onto a CCA plate and use a hockey stick to spread out 2. Inoculate 50ul onto a CNA plate and plate for single colonies 3. Dilute neat sample 1:100 (0.1 ml + 9.9ml PBS). Inoculate 50ul onto a CBA and use a hockey stick to spread out. Incubate CBA CO_2 and CCA/CNA O_2			
Financial barriers			
No.			
Health benefits			
No.			

Recommended action	ACCEPT The document now has a more detailed method.

Comment number	5		
Date received	20/01/2014	Lab name	University of Iceland
Section	Various		
Comment			

a. Introduction

Change: Usually these give rise from the colonising oral flora but can also result from a flare-up of a chronic low-grade infection.

To: Usually these arise from the colonising oral flora but can also result from a flare-up of a chronic low-grade infection.

b. Introduction - Oral Mucositis

Oral mucositis is a painful complication of chemotherapy or head and neck radiotherapy, caused by direct cytotoxicity of the treatment regime. Super-infection, usually with yeasts and oral bacteria, can exacerbate the problem and microbiological examination can help to guide symptomatic treatment.

c. Erythematous and Pseudomembranous Candidosis

Atrophic candidosis (denture stomatitis) may occur in the palatal mucosa below the fitting surface of dentures, especially when patients sleep with their dentures in place &/ or have xerostomia.

d. Angular Cheilitis and Peri-Oral Infections

It is common for dentate patients with angular cheilitis to have infection with both *S. aureus* and *C. albicans* in the labial commissure region.

e. 4.5 Culture and Investigation

Saliva samples may be collected for microbiological investigation and for other types of assessment. Increasingly saliva is being used as a sample for new diagnostic techniques, but also for assessing xerostomia and caries risk Care is needs to avoid contamination of these specimens and cross infection from these specimens. Sometimes culture is done with an exact volume of saliva in order to assess the count of a particular organism (eg *S. mutans* or lactobacilli per mL of the original saliva sample.

f. 5.1 Microscopy

Perhaps this is still useful for diagnosis acute necrotizing ulcerative gingivitis, not least in a suspected HIV positive patient.

Recommended action	a. **ACCEPT** b. **ACCEPT**

	c. **ACCEPT** d. **ACCEPT** e. **ACCEPT** f. **NONE** Gingivitis is not covered in this SMI.

Comment number	6		
Date received	20/01/2014	**Lab name**	PHE, Mycology Reference Laboratory
Section	Introduction, 1.3, 4.4, 4.5, 4.6, 4.9, 5 and appendix		
Comment			

Final comments from the UKCMN

a. Introduction Infection of Salivary Glands Should the SMI covering these infections be referenced in this section? In the subsequent section there is the number and name of the SOP covering the process.

b. 'Non-albicans Candida species and non-albicans yeasts should be described as Candida species other than C. albicans or Yeasts other than C. albicans throughout.

c. Section 1.3 - Statement needed re containment level 3 for Histoplasma (and other relevant dimorphic pathogens causing oral ulceration) risk.

d. Section 4.4 - A comment is needed under the microscopy section to suggest that direct microscopic examination with Calcofluor staining may be helpful if Histoplasma or mould infection is suspected.

e. Section 4.5 referred to SOP Q 5 “Inoculation of culture media”. Section 4.3 of this SOP recommends centrifugation for liquid specimens prior to plating with a loop but does not cover the quantitative procedure for oral rinses as set out in the Introduction under erythematous and pseudomembranous candidosis. Should a quantitative or, more appropriately, a semi quantitative procedure be added (ie scanty, +, ++, or +++).

f. Section 5 - reporting procedure. Although the introduction mentions quantitative culture of oral rinses the quantitative aspect is not followed up in Section 5.

g. Section 4.5.1 plates are only kept for 40-48 hours. This would not be long enough for Histoplasma to grow. Histoplasmosis is mentioned under oral ulceration so there needs to be a proviso for extending the incubation (under cat 3 conditions) if this is suspected. This incubation time might also be too short for potential moulds to develop if they are suspected. Incubation temperature of 35-37C might be too high for some of the rarer moulds causing palate infection. Perhaps if an unusual infection is suspected a second Sab plate should be set up at 30C. Rarer moulds were referred to under the section describing erythematous and pseudomembranous candidosis so perhaps an addition is needed here.

h. Section 4.6.1 - links to SMIs for organism identification do not lead to any

recommendations for either Candida or Aspergillus and some organisms aren't linked at all! i. Section 4.9 line 7 - small typo nation should be national. j. Appendix - amend the flow chart in the appendix to include longer incubation on Sab agar to recover moulds and Histoplasma if suspected. A statement could be added regarding Sab culture at 30°C if invasive mould infection is suspected and possible identification.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **ACCEPT** The relevant documents are currently under review and being restructured once it is known which document should be cross referenced it will be inserted. b. **ACCEPT** The changes have been made. c. **ACCEPT** Sentence has been inserted. d. **ACCEPT** The section has been amended. e. **ACCEPT** This information has been included. f. **ACCEPT** g. **ACCEPT** h. **ACCEPT** Where there are documents that can be cross referenced hyperlinks have been inserted. Where documents are missing from the repository a decision will be made as to whether they need to be written. i. **ACCEPT** j. **ACCEPT**

3rd Consultation: 02/06/2014 – 26/08/2014

Version of document consulted on: B 4do+

Proposal for changes

Comment number	1		
Date received	10/06/2014	**Lab name**	Princess of Wales' Hospital
Section	All		
Comment			
No changes/comments for consideration for submission.			
Recommended action	**NONE**		

Comment number	2		
Date received	01/08/2014	**Lab name**	Truro
Section	Page 16 and page 12		
Comment			
Page 16 - The use of blood agar should be dictated by appropriate clinical details on request not a standard for every mouth swab as indicated on the chart on page 12.			
Recommended action	**NONE** Certain organisms are easier to recognise on a blood agar plate.		

Comment number	3		
Date received	12/08/2014	**Lab name**	PHE, Cambridge
Section	Sections 2-4		
Comment			
a. The SMI notes that saliva and oral rinses are increasingly used as a method of microbiological investigation including to distinguish between colonisation or infection. Reporting is on clinically significant isolates or presence or absence of growth. Are there any recommendations on the quantitative or semi-quantitative reporting of cultures. b. We currently do not culture or report the presence of coliforms, non-fermenters in immunocompromised patients with oral mucositis unless specifically requested. If routinely performed, it will lead to a large increase in workload and we are not sure of the clinical benefit. c. We currently report the presence of yeasts in mouth swabs and identify yeasts to			

species level and perform susceptibility on clinical request usually when there is failure to respond to conventional therapy. The majority are likely to be Candida. Identifying all yeasts to species level will increase workload with unclear patient benefit. d. Vincent's stain - this has not been mentioned - is this recommended elsewhere?
Evidence
Cochrane Database Syst Rev. 2010 Aug 4;(8):CD001973. doi: 10.1002/14651858.CD001973.pub4.Interventions for treating oral mucositis for patients with cancer receiving treatment. Clarkson JE1, Worthington HV, Furness S, McCabe M, Khalid T, Meyer S.
Financial barriers
Financial barriers as above.

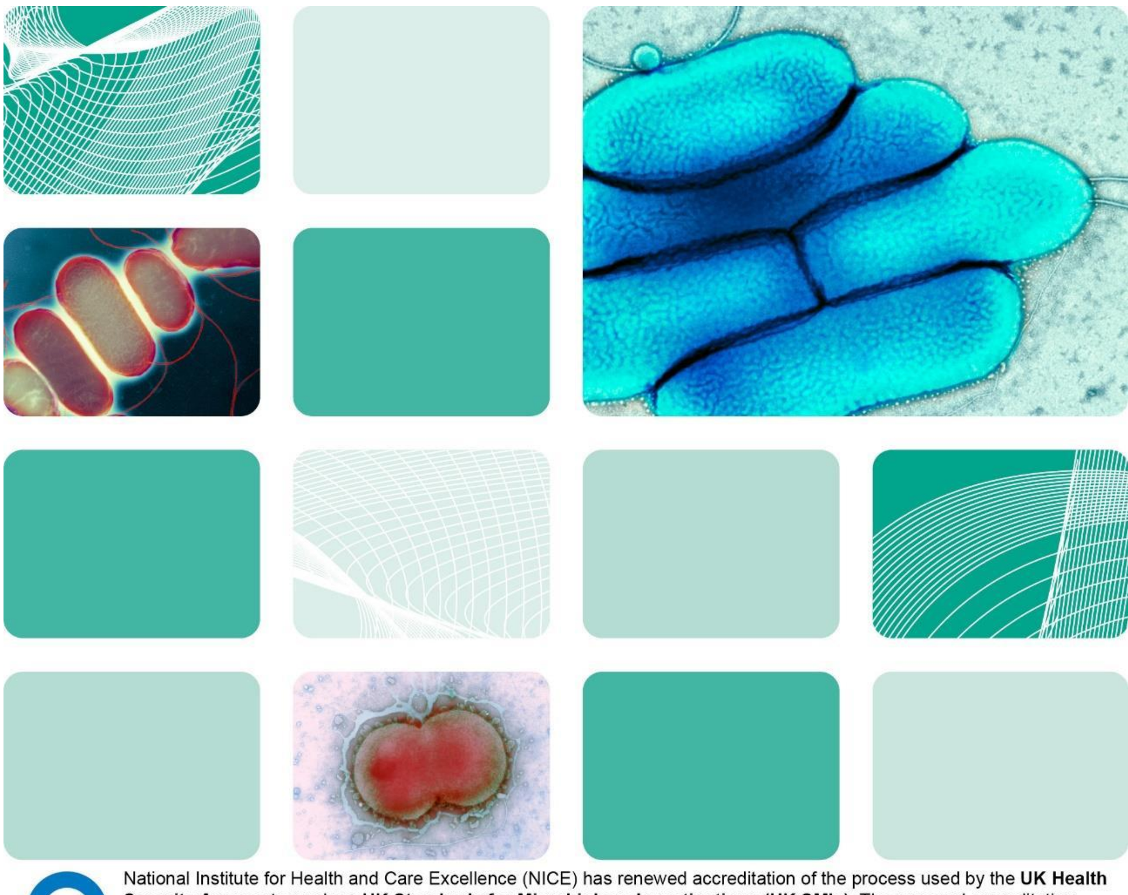
Recommended action	a. **ACCEPT** This information has been inserted in to the document. b. **ACCEPT** The document has been amended to reflect this. c. **ACCEPT** The document has been amended to reflect this. d. **ACCEPT** This is referred to in the introduction.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 7			
Date received	02/04/2013	**Lab name**	Dept of Clinical Microbiology, Royal Cornwall Hospital
Date received	05/04/2013	**Lab name**	Bristol
Date received	18/04/2013	**Lab name**	Golden Jubliee National Hospital
Date received	13/05/2013	**Lab name**	Spire Pathology Services
Date received	16/01/2014	**Lab name**	Golden Jubilee National Hospital
Date received	19/01/2014	**Lab name**	Sunderland
Date received	02/06/2014	**Lab name**	ex microbiology Careggi Firenze

UK Standards for Microbiology Investigations

Investigation of samples from paranasal sinuses



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

Issued by the Standards Unit, Specialised Microbiology and Laboratories, UKHSA

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:

Applied Microbiology International

Displayed logos correct as of June 2024.

Contents

Acknowledgments 2

Contents 3

Amendment table 4

1 General information 6

2 Scientific information 6

3 Scope of document 6

4 Introduction 7

5 Safety considerations 10

6 Pre-laboratory processes (pre analytical stage) 11

7 Laboratory processes (analytical stage) 12

8 Post-laboratory processes (post analytical stage) 19

9 Antimicrobial susceptibility testing 22

10 Referral to reference or specialist testing laboratories 22

Algorithm: Investigation of samples from paranasal sinuses 23

References 24

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	12/16.09.24
Issue number discarded	7.1
Insert issue number	8
Anticipated next review date*	16.09.27
Section(s) involved	**Amendment**
Title	The title has been changed from 'Investigation of nasal samples' to 'Investigation of samples from paranasal sinuses'.
Whole document	Hyperlinks updated to direct reader from UK SMIs webpages on GOV.UK to RCPath website. The term 'Sinusitis' has been replaced with 'Rhinosinusitis' throughout the document. Subheadings have been revised and modified where needed. All sections have been updated with current and relevant information and supported with recent literature where available. Some sections have been restructured as appropriate to align with current laboratory practices.
Scope of document	Types of specimens have been moved to Section 6: Pre-laboratory processes (pre analytical stage). Other diagnostic methods are considered within the scope but not detailed in the document, emphasising that the diagnosis of rhinosinusitis involves multiple approaches. Topics that are outside the scope of this document have been mentioned and links to relevant UK SMIs were provided if available. The reader is also made aware of the updated taxonomy of Candida species.

Section 4: Introduction	Information updated and supported with recent literature where available. Section on nasal carriage has been removed and the link to UK SMI B 29: Investigation of specimens for screening for MRSA has been provided in the scope.
Technical Information/Limitations	This section has been removed. Information replaced with a link to refer reader to scientific information. Information regarding limitations of diagnostic methods has been incorporated into the relevant sections specific to each method.
Table 1. Culture media, conditions, and organisms	Table has been reviewed and updated.
Table 2. Minimum level of identification in the laboratory	Table has been reviewed and updated.
Section: 7 Laboratory processes (analytical stage)	Sections 7.5 molecular assays and 7.6 Antigen/antibody testing have been added.
Section 9: Antimicrobial susceptibility testing	Information and hyperlinks reviewed and updated as appropriate. Hyperlink to British Society for Antimicrobial Chemotherapy (BSAC) removed. Hyperlink to Clinical and Laboratory Standards Institute (CLSI) added.
Section 10: Referral to reference or specialist testing laboratories	Hyperlinks were updated as appropriate.
Algorithm	The content of the algorithm has been reviewed and updated to align with the document.
References	References reviewed and updated.

*Reviews can be extended up to 5 years where appropriate.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMIs) document describes the examination of sinus aspirates and associated samples for the detection and recovery of the organisms that cause the various forms of rhinosinusitis. This document does not cover investigations for conditions of viral, allergic or toxin origin.

Other diagnostic techniques such as Computed Tomography (CT) scans are commonly employed by healthcare professional for the diagnosis of rhinosinusitis and should be considered alongside the microbial methods mentioned in this document.

Please refer to UK SMI B 29 - Investigation of Specimens for Screening for MRSA for information on screening for MRSA nasal carriage.

Please note the following:

This document does not provide the details for the identification of organisms causing rhinosinusitis. The identification section in this document only provides information on the level to which organisms should be identified to as part of the reporting process with direct links to the relevant identification documents for reference.

This document addresses samples sent to clinical laboratories from both primary and secondary (hospital) healthcare. However, most cases of rhinosinusitis are diagnosed and managed in primary healthcare settings without the necessity of sending samples to clinical laboratories. Also, most cases are uncomplicated and tend to resolve on their own without further investigations or antimicrobial treatment. For more information, refer to the NICE guideline on Sinusitis (acute): antimicrobial prescribing and to their website for information on the management of acute and chronic rhinosinusitis and prescribing information.

The advancement of molecular phylogenetic analysis has greatly influenced the taxonomy of microorganisms, especially fungi. This has resulted in the reclassification and renaming of many species. For the purpose of this document, both the previous and current nomenclature of reclassified species or species with updated nomenclature will be mentioned, as required. This also applies to the species formerly part of the genus Candida and will collectively be referred to as '*Candida* and associated ascomycetous yeast' (1).

This UK SMI should be used in conjunction with other relevant UK SMIs.

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4 Introduction

4.1 Rhinosinusitis

Rhinosinusitis refers to inflammation in the nasal mucosa and one or more of the paranasal sinuses: maxillary, ethmoid, frontal and sphenoid, with the maxillary being the commonest sinus to get infected (2). It can result from viral, bacterial, or fungal infections (2,3). Clinical presentations of rhinosinusitis are diverse and can overlap with other conditions such as hay fever. Symptoms include nasal congestion and discharge, facial pain, pressure or fullness, headaches and fatigue. Factors that predispose an individual to rhinosinusitis include impaired mucociliary function, obstruction of the sinus entrance (e.g., by nasotracheal intubation or by mucosal oedema as a result of viral infection) and defects in the immune system (4-6). The sinus cavities are usually sterile or may contain small numbers of bacteria that are continuously removed by the mucociliary system.

Rhinosinusitis aspiration is generally the preferred method for specimen collection as it allows more targeted sampling and reduces risk of contamination by upper respiratory tract flora (2,7). Specimens should be collected by Ear, Nose and Throat (ENT) surgeons and obtained through rigid endoscopy and careful aspiration of the sinus cavity avoiding contamination by upper respiratory tract flora.

In the case of suspected invasive fungal rhinosinusitis, a biopsy sample may also be obtained for direct microscopic examination prior to culture to identify the presence of fungal elements (8,9).

4.1.1 Acute rhinosinusitis

Acute rhinosinusitis is often suspected if the symptoms meet clinical criteria and deteriorate after 5 days or persist beyond 10 days (less than 12 weeks) (6). The aetiology of community acquired infections can be viral, bacterial, mixed (viral and bacterial) or occasionally fungal (particularly in immunosuppressed patients) with viral upper respiratory tract infections being the most common cause of acute rhinosinusitis.

For acute rhinosinusitis, routine culture and antibiotic prescription is not recommended as acute rhinosinusitis often resolves on its own without the need for antibiotics (7,10).

Culture is selectively obtained in individuals at high risk of complications or antibiotic resistance. Antibiotic prescription is often reserved for patients with persistent or worsening symptoms or complications of acute rhinosinusitis (10). Serious complications of acute rhinosinusitis resulting in the formation of abscess such as subperiosteal abscess or brain abscess may also require surgical intervention and drainage.

Community rhinosinusitis

The most common bacteria isolated from cases of acute community acquired rhinosinusitis are *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* which is more prevalent in children than adults (2,11,12). Other less common organisms isolated are streptococci of the “anginosus” group (*Streptococcus anginosus, Streptococcus constellatus* and *Streptococcus*

intermedius), group A streptococcus, other α-haemolytic streptococci, *Staphylococcus aureus* and anaerobic bacteria (which are infrequent in children) (2,13).

Nosocomial rhinosinusitis

Nosocomial rhinosinusitis is often a complication in endotracheal intubation and mechanical ventilation and often shows no clinical signs of infection (14,15). The causative organisms can vary and infections are often polymicrobial. *Pseudomonas aeruginosa* and other Gram-negative rods commonly cause nosocomial rhinosinusitis in intensive care and immunocompromised patients (2,16). Similar pathogens and polymicrobial infections have been identified in children with more anaerobes being isolated (17,18). Acute fungal rhinosinusitis caused by *Aspergillus* or Mucorales species can occur in immunosuppressed patients, such as haematology patients undergoing chemotherapy, allogeneic or solid-organ transplantation recipients and patients with poorly controlled diabetes.

Nosocomial infections are often underestimated and difficult to diagnose in intensive care patients, therefore close collaboration among physicians, ENT surgeons, microbiologists and histopathologists is necessary to reach a diagnosis.

4.1.2 Chronic rhinosinusitis

Chronic rhinosinusitis is a long-term inflammation of the sinuses that last for longer than 12 weeks without complete resolution (6). Chronic rhinosinusitis is multifactorial in nature and can include allergies, anatomical abnormalities, environmental irritants, and infections including odontogenic infections and those associated with biomaterials such as dental implants. It may also be a feature of some congenital immunodeficiency syndromes and disorders of mucociliary function, although most patients do not have these conditions. In addition, chronic rhinosinusitis can persist in some patients even after surgical intervention.

Chronic rhinosinusitis is clinically sub-categorised by the presence or absence of nasal polyps (CRSwNP or CRSsNP respectively) (5,6). Further research and understanding of chronic rhinosinusitis have led to the proposal of additional categorisation models to enhance the accuracy of diagnosis and management of different subgroups of patients (5,6,19,20).

Mucosal inflammation in chronic rhinosinusitis can persist based on the immune responses. Type 2 immune response can lead to persistent mucosal inflammation and is a characteristic of CRSwNP, especially in western countries. Type 1 inflammation is more commonly associated with CRSsNP. Biofilm formation by microorganisms in the sinonasal cavity can exacerbate Type 1 inflammation, contributing to persistent infections (5). Biofilms are difficult to treat and often necessitate surgical intervention.

The microbiology of chronic rhinosinusitis differs significantly from that of acute rhinosinusitis and is commonly caused by anaerobes, Gram-negative bacteria, *Staphylococcus aureus*, and fungi (usually *Aspergillus* species and occasionally other moulds). The persistence of infection or extension of unresolved acute rhinosinusitis can lead to a shift in the types of bacteria present from aerobic to anaerobic bacteria. This includes *Pseudomonas aeruginosa*, particularly from patients with cystic fibrosis, *Enterobacterales*, *Peptostreptococcus* species, *Cutibacterium* (Formerly

Propionibacterium) species, *Fusobacterium* species, *Prevotella* species and other anaerobic Gram-negative bacteria (21-24).

Chronic rhinosinusitis can lead to serious and life-threatening complications with the most common being orbital infections (25-27). Intracranial infections are less common but may cause significant morbidity and mortality. Another rare complication is osteomyelitis (see UK SMI B 42 - Investigation of Bone), involving the frontal bone (Pott's puffy tumour). *Staphylococcus aureus* and anaerobes are the predominant isolates from such cases and are also recovered from children with severe rhinosinusitis symptoms requiring surgical intervention or with protracted rhinosinusitis (lasting over one year) (12).

4.1.3 Fungal rhinosinusitis

Fungal rhinosinusitis can be acute or chronic and is categorised into non-invasive and invasive fungal rhinosinusitis depending on the extent of fungal involvement in the sinuses and the severity of the infection. Non-invasive fungal rhinosinusitis may take the form of a fungus ball in the sinus, saprophytic fungal rhinosinusitis or allergic fungal rhinosinusitis (28-30). Invasive fungal rhinosinusitis can be either acute or chronic and may form granulomas within the sinus cavities. Granulomatous invasive rhinosinusitis may be confused with Granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis) or squamous cell carcinoma (28). Therefore, examination of tissue rather than pus is important in fungal rhinosinusitis and close co-operation among the surgeon, microbiologist and histopathologist is also necessary (8,28). Acute invasive rhinosinusitis can spread rapidly from the involved sinuses and is to be regarded as a medical emergency. Aggressive surgical debridement is often required in addition to systematic antifungal therapy and treatment of the underlying cause.

Fungal rhinosinusitis is predominantly caused by moulds, with *Aspergillus* species being the most common pathogens. Other moulds that are less commonly encountered include Mucor and Rhizopus which are associated with the fatal invasive fungal infection, mucormycosis (31,32). Also, less common are thermally dimorphic fungi such as *Sporothrix schenckii*, one of the causative agents of endemic mycoses. These exist as tissue invasive yeast at human body temperature and as moulds in the environment (33). They are limited by geographical distribution and rare in the UK.

Community-acquired chronic fungal rhinosinusitis is a relatively common problem in some tropical and subtropical countries which can in some instances lead to invasive disease, e.g., in Africa and India, and imported cases may be encountered (32). The commonest cause overall is *Aspergillus flavus*, but other fungi should be considered.

It is important to take into consideration the country of origin and travel history of patients in suspected cases of fungal rhinosinusitis to help determine the causative agent of infection. Rhino-orbital cerebral mucormycosis, which became more prevalent in India during COVID-19 pandemic, highlights the importance of such considerations in diagnosis and treatment planning (34).

5 Safety considerations

The section covers specific safety considerations (35-55) related to this UK SMI, and should be read in conjunction with the general safety considerations.

5.1 Specimen collection, transport and storage:

Use aseptic technique.

Collect all specimens before antimicrobial or antifungal therapy where possible.

Collect specimens in appropriate CE marked leak-proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

5.2 Specimen handling, processing and examination:

The majority of diagnostic work can be conducted at Containment Level 2. However, specific procedures involving mycology work and certain hazard group 2 bacterial isolates should be conducted within a microbiology safety cabinet.

When Hazard Group 3 organisms are suspected, or clinical details regarding travel history are not available for a risk assessment, all specimens must be processed in a microbiology safety cabinet under full containment level 3 conditions. General containment level 3 procedures are also recommended for the examination of cultures that may contain dimorphic fungi and other pathogenic fungi. Sealed containers such as screw-capped bottles should be used for culture. Culture plates are not suitable.

Any laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

6 Pre-laboratory processes (pre analytical stage)

6.1 Specimen type:

Antral washout, sinus aspirate, sinus washout, and tissue.

Notes:

Nose swabs are not a suitable sample type for the diagnosis of rhinosinusitis although they can be used for screening for nasal carriage of e.g., MRSA or *Candida auris*. However, superficial nasal suction samples and swabs can be used to investigate nasal discharge in patients with nasogastric tube or post ENT surgery.

Endoscopic aspiration is recommended for areas that are challenging to access directly and for reducing the risk of iatrogenic trauma and blood contamination of the samples that may be caused by blind aspiration (56).

The choice of specimen is influenced by the patients' clinical details and the investigation aim. For example, sinus aspiration may be sufficient for the diagnosis of bacterial rhinosinusitis while additional biopsy may be required for the identification of fungal structure and more detailed analysis of tissue changes.

6.2 Specimen collection and handling:

For safety considerations refer to Section 5.

Collect specimens before antimicrobial therapy where possible.

Collect specimens other than swabs into appropriate containers and place in sealed plastic bags.

Ideally, a minimum volume of 1mL for washouts is required to ensure adequate sensitivity.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

6.3 Specimen transport and storage:

Specimens should be transported and processed as soon as possible.

The volume of specimen influences the transport time that is acceptable. Large volumes of purulent material maintain the viability of anaerobes for longer.

The recovery of fastidious organisms and anaerobes in particular is compromised if the transport time is delayed.

Samples should be kept at room temperature if they are to be processed immediately. Otherwise, refrigeration is preferable to storage at ambient temperature.

7 Laboratory processes (analytical stage)

7.1 Specimen processing:

For safety considerations refer to Section 5.

Standard

Non-mucoid sinus or antral washouts are processed as follows:

- Centrifuge specimen (for antral washouts), unless very mucoid, at 1200 x *g* for 10 minutes
- Discard most of the supernatant, leaving approximately 0.5mL
- Resuspend the centrifuged deposit in the remaining fluid
- Carry out microscopy and culture

Mucoid specimens are processed by digestion as follows:

- Carry out microscopy
- Add equal volume of a 0.1% solution of N-acetyl cysteine to specimen
- Agitate gently for approximately 10 seconds
- Incubation at 35-37°C for 15 minutes followed by gentle agitation for approximately 15 seconds will assist homogenisation

  Note: do not exceed 20 minutes of incubation to avoid overprocessing the sample.
- Inoculate plates

7.2 Microscopy

Refer to UK SMI TP 39 – Staining procedures

For information on technical limitations refer to scientific information.

Direct microscopic examination of fresh or stained specimens can provide pre-diagnostic information on the presence of fungal structures as culture results may not be available for a few days. This is particularly important for the prompt administration of anti-fungal therapy in immunocompromised patients. However, this method is insensitive and negative results do not rule out a fungal infection as may be the case in the early stages of the infection.

Standard

For mucoid specimens:

Using a sterile loop select the most purulent or blood-stained portion of the specimen and make a thin smear on a clean microscope slide for Gram staining.

For non-mucoid specimens:

Using a sterile pipette place one drop of centrifuged deposit (see section 6.1.2) or neat specimen on to a clean microscope slide. Spread this with a sterile loop to make a thin smear for Gram staining.

Note: If fungal infection is suspected or seen in the Gram stain carry out the supplementary examination.

Supplementary

Using a sterile pipette, place one drop of centrifuged deposit (see section 6.1.2) or neat specimen on a clean microscope slide.

Examine at 10x and 40x magnification using potassium hydroxide (KOH) or enhanced with calcofluor white or blankophor white staining for fungal hyphae.

7.3 Culture

Using a sterile loop inoculate each agar plate with centrifuged deposit (see UK SMI Q 5 - Inoculation of Culture Media for Bacteriology).

For fungal culture on Sabouraud slopes use a pastette to place 2 - 3 drops of deposit onto the agar.

Table 1: Culture media, conditions, and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Rhinosinusitis	Antral washout/ Sinus aspirate/ Sinus washout	Blood agar and / or Staph / Strep selective agar	35 to 37 35 to 37	5 to 10 % CO_2 Air	40 to 48hrs 40 to 48hrs	daily daily	**Common pathogens:** *M. catarrhalis* *S. pneumoniae* *S. aureus* Lancefield Groups A, C, G and F Streptococcus anginosus group Other organisms in pure growth may be significant.

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Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Rhinosinusitis	Antral washout/ Sinus aspirate/ Sinus washout	Chocolate agar with or without bacitracin[a]	35 to 37	5 to 10% CO_2	40 to 48hrs	daily	**Common Pathogens:** β-haemolytic streptococci Enterobacterales[b] *H. influenzae* *M. catarrhalis* Pseudomonads *S. aureus* *S. anginosis* group *S. pneumoniae*
		Neomycin fastidious anaerobe agar with 5 µg metronidazole disc	35 to 37	Anaerobic	48hrs or 5 to 7 days[c]	48hrs and at 5 to 7 days[c]	**Common Pathogens:** *Fusobacterium* species *Peptostreptococus* species *Cutibacterium* species *Prevotella* species

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Chronic rhinosinusitis or Rhinosinusitis in immunocompromised patients[d]	Antral washout/ Sinus aspirate/ Sinus washout/ Tissue[e]	Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin[f]	30 to 37[g]	Air	5 to 7 days[h]	Greater than 40 hrs and at 5 to 7 days[h]	Moulds[i]

a May include either a bacitracin 10-unit disc or bacitracin incorporated in the agar. When bacitracin is incorporated into the plate, a separate blood agar plate incubated in 5 to 10% CO_2 will need to be put up to detect *M. catarrhalis* and *S. pneumoniae*.

b Gram-negative selective plate (CLED or MacConkey) could be added for the isolation and differentiation of Gram-negative organisms, including those in the Enterobacterales order.

c Review the plates at 48 hours. If there is no growth and there is a clinical suspicion of slow-growing facultative anaerobes, such as *Cutibacterium acnes* or *Actinomyces* species then re-incubate for 5 to 7 days.

d Immunocompromised patients groups include haematology, oncology, diabetes (uncontrolled), neutropenic (other).

e When testing tissue biopsies, specimens should not be overly processed (e.g., crushed) as this may damage hyphal elements and minimise the chances for isolating the fungus.

f Supplemented with chloramphenicol or gentamicin; chromogenic media may be useful for identifying mixed fungal infections.

g In case of suspected mucormycosis or observation of hyphae of Mucorales in direct microscopy, it is recommended to incubate the cultures at two separate temperatures, 30 °C and 37 °C to increase the yield.

h Moulds are the main fungal pathogens for rhinosinusitis whilst yeast growth normally represents colonisation. Therefore, extended incubation is advocated for a minimum of 5 to 7 days, for all surgical samples and for the investigation of fungal rhinosinusitis in high-risk patients. Plates should be read after 40 hours and at 5 to 7 days. Some fungal pathogens may require extended incubation up to 14 days.

If based on travel history endemic mycoses such as histoplasmosis is suspected, samples should be processed in containment level 3 and all yeasts identified to species level.

i Differentiation between yeast and filamentous forms may require microscopic examination. Wet mounts or stain with lactophenol cotton blue (LPCB) should be used.

For fungal culture, one SAB with chloramphenicol or gentamicin plate should be used per sample and streaked as per routine and standard bacteriology practice. However, increasing the number of plates will increase the sensitivity. It is highly recommended that SAB with chloramphenicol or gentamicin plates be sealed with gas-permeable tape or alternatively placed inside a sealable plastic bag during incubation to avoid cross contamination. Incubation of SAB with chloramphenicol or gentamicin plates in ‘automated incubation and imaging’ modules may lead to fungal contamination of modules and other cultures. No fungal isolate should be dismissed as a ‘contaminant’ without full identification.

If microscopy is suggestive of organisms not listed in the target list additional and/or other media may be required.

Laboratory should follow local protocols or manufacturer’s instructions for optimal incubation conditions.

7.4 Identification

Refer to individual UK SMIs for organism identification.

Table 2. Minimum level of identification in the laboratory

Target organism	Identification level
Peptostreptococcus species	"anaerobes" level
Cutibacterium species	"anaerobes" level
Fusobacterium species	"anaerobes" level
Prevotella species	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Enterobacteriaceae	species level
Fungi*	species level (if clinically significant)
H. influenzae	species level
M. catarrhalis	species level
Pseudomonas species	species level
S. aureus	species level
S. anginosus	*S. anginosus* group level
S. pneumoniae	species level

* Moulds are more commonly associated with rhinosinusitis.

Note: Any organism considered to be a contaminant may not require identification to species level. Organisms may be identified further if clinically or epidemiologically indicated.

7.5 Molecular assays

Molecular methods such as Nucleic Acid Amplification Test (NAAT) and fluorescence *in situ* hybridization have been developed for the rapid detection of fungal species directly from patient specimen. These assays allow for the timely diagnosis of invasive fungal rhinosinusitis from tissue samples and subsequently the prompt initiation of antifungal therapy (57). This is crucial due to the high risk of complications and mortality associated with these types of infections in immunocompromised and diabetic patients. In addition, molecular methods can help overcome some of the challenges encountered with culture-based methods such as false negative results and the identification of rare fungal species (57,58).

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There are numerous commercially available assays that have been developed for the detection of fungal organisms ranging from pan fungal to pathogen specific (e.g., *Aspergillus,* Mucorales) assays, with pathogen specific assays having higher sensitivity and faster turnaround time (59,60). The quality of these assays is largely dependent on the analytical range of detection provided by the specific assay, type and volume of specimen, the DNA fungal extraction method and automation of the process to increase sensitivity (60,61). Currently, the validation of NAAT for the diagnosis of fungal rhinosinusitis is relatively limited and it is essential that these assays are validated for the specimen type before their use in laboratories.

Despite the advancement in molecular methods, they are not routinely used for the detection of fungal rhinosinusitis and have not yet replaced culture-based methods in clinical laboratories. These methods would often be used in conjunction with culture-based methods as appropriate or utilised largely by reference laboratories (57,60). Where fungal elements are visualized in specimens but are culture negative, molecular testing may be a useful adjunct test to provide identification (61).

7.6 Antigen/antibody testing

Antigen/antibody tests have many limitations and are not regularly used as diagnostic tools for rhinosinusitis, including invasive fungal rhinosinusitis. Factors that can influence the performance of these assays include variation in sensitivity and specificity depending on the clinical setting e.g., underlying conditions and immunosuppression as well as false positive results due to cross-reactivity e.g., individuals with yeast colonisation (62-64).

Furthermore, antibody tests have limited applicability in the diagnosis of invasive fungal rhinosinusitis, because of the reduced or delayed antibody production in immunocompromised individuals.

8 Post-laboratory processes (post analytical stage)

8.1 Microscopy

8.1.1 Reporting microscopy

Report microscopy results as:

Gram’s stain

- Report presence of WBCs.
- Report if organisms detected.

Note: The presence of yeast cells e.g., *Candida* is very rare and usually a reflection of oropharyngeal colonisation. In such cases further histopathological evidence is required.

Fungal stain

- Report presence or absence of fungal elements.
- Differentiate between yeasts and filamentous fungi (moulds).
- Where possible provide a description of the filamentous fungi/element observed.

Notes:

- The presence of fungal structures in biopsy specimens from deep tissues should be reported as clear/proven evidence of fungal infection.

- The presence of broad, aseptate or pauci-septate hyphae with wide-angle branching is consistent with Mucorales. The presence of regularly septate hyphae with 45° branching is consistent with *Aspergillus* species. but could represent other hyaline fungi such as *Scedosporium* species.

- Reports simply stating fungal elements seen with no differentiation, are of limited clinical utility and should be avoided.

8.1.2 Microscopy reporting time

Interim or preliminary results should be issued on detection of clinically significant results as soon as growth is detected unless specific alternative arrangements have been made with the requestors.

In immunocompromised patients or when fungal investigation is specifically requested, microscopy positive fungal results indicating presence of filamentous hyphae indicative of mucoraceous mould (members of Mucorales) or *Aspergillus species* should be immediately communicated to the consultant looking after the patient or an infection consultant liaising with the clinical teams.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer-generated reports should follow preliminary and verbal reports as soon as possible.

8.2 Culture

8.2.1 Reporting Culture

Report culture results as:

Bacterial Culture

- Clinically significant organisms with antimicrobial susceptibility results
- No growth of clinically significant organisms*
- No growth

* Identification should not be reported for organisms of no clinical significance.

Fungal culture

- Yeasts should be reported along with an indication of growth quantity of scantly/light, moderate or heavy to allow for interpretation of significance.
- Isolation of any filamentous fungi should be reported, irrespective of burden.
- No fungal growth.

Notes

- Fungal growth may be reported as negative after 48 hours incubation although cultures will continue to be incubated up to 5 - 7 days and extended culture (up to 14 days may be required where fungal rhinosinusitis is specifically suspected). In the event of fungal growth, a further report will be issued.
- The presence of fungi should be documented even when a fungal culture is overgrown by chloramphenicol-resistant Gram-negative bacterial (e.g., *Pseudomonas* spp.). This should be noted in the result and not reported as 'fungi not isolated'.
- All clinically significant fungal isolates should be identified to species level (for yeast species, level identification is essential in recurrent or recalcitrant infections).

8.2.2 Culture reporting time

Interim or preliminary results should be issued on detection of clinically significant isolates as soon as growth is detected unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer-generated reports should follow preliminary and verbal reports as soon as possible.

See appropriate UK SMIs for supplementary investigations.

8.3 Reporting other tests including molecular testing

As newer and more novel methods are becoming available, their validation and reporting should follow local protocols or manufacturer's instructions.

9 Antimicrobial susceptibility testing

For interpretation of susceptibility testing results, laboratories should test and interpret according to the EUCAST breakpoint, refer to EUCAST guidelines for breakpoint information.

Alternatively, the Clinical and Laboratory Standards Institute (CLSI) method along with the corresponding CLSI breakpoints can be used: Susceptibility Testing Subcommittees (clsi.org).

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

Antifungal susceptibility testing should be performed for fungal rhinosinusitis, when a culture is available.

9.1 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

10 Referral to reference or specialist testing laboratories

In case of sending away isolates to reference or specialist testing laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratories for sending isolates.

Contact the appropriate reference laboratory (refer to the links provided below) for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission.

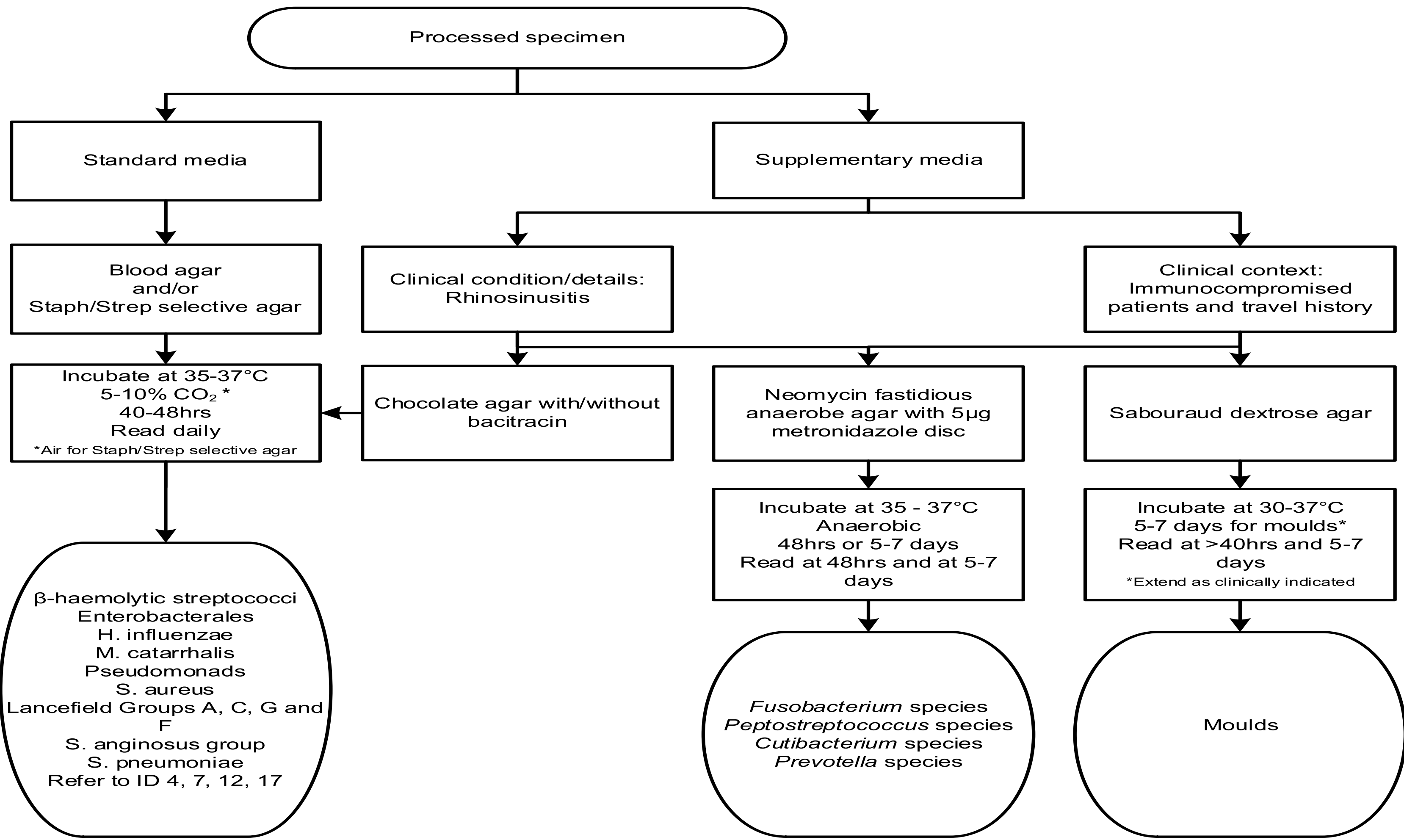
England

Wales

Scotland

Northern Ireland

Algorithm: Investigation of samples from paranasal sinuses



Please see Table 1 for algorithm details. If microscopy is suggestive of organisms not listed in target list, additional and/or other media may be required. In addition, if fungal elements are seen but the culture is negative, molecular identification may be useful.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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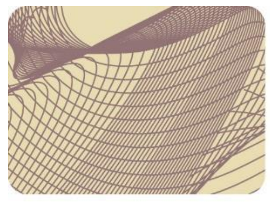
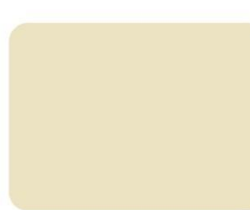
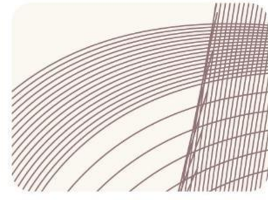
UK Standards for Microbiology Investigations

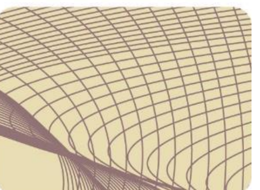
Review of users' comments received by Working group for microbiology standards in clinical bacteriology

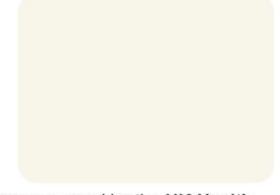
B 05 Investigation of samples from paranasal sinuses











National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

This publication was created by UK Health Security Agency (UKHSA) in partnership with the partner organisations.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

Issued by the Standards Unit, Specialised Microbiology and Laboratories, UKHSA

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Consultation: 07/05/2024 – 24/05/2024
Version of document consulted on: dl +

Title

Comment number: 1

Date received: 07/05/2024
Laboratory or organisation name: Southwest Pathology Services

Would you consider appending the title to include Antral Washout or AWO? Although SOP later states that Nasal swabs are unsuitable specimens I feel that differentiating this at SOP title level will make this caveat crystal clear.

Recommended action

1. Accept: The title has been changed from 'nasal and paranasal sinus samples' to 'Investigation of samples from paranasal sinuses'.

4 Introduction

Comment number: 2

Date received: 07/05/2024
Laboratory or organisation name: Southwest Pathology Services

What about mentioning Klebsiella rhinoscleromatis? Can cause symptoms very similar to sinusitis

Recommended action

1. None: *Klebsiella rhinoscleromatis* is uncommon in developed countries. Only common/clinically relevant species are listed in UK SMIs.

7.3 Culture

Comment number: 3

Date received: 07/05/2024
Laboratory or organisation name: Southwest Pathology Services

I would expect to see the addition of a gram-negative selective plate (CLED or MacConkey) for Enterobacterales - this group of organisms has certainly been associated with symptoms of chronic sinusitis - especially if from a dental or maxillary source.

Recommended action

1. Accept: The table footnotes have been updated with this information.

Comment number: 4

Date received: 07/05/2024
Laboratory or organisation name: UK Health Security Agency in collaboration with North Bristol NHS Trust

In the table, under Common organisms - Enterobacteriaceae should be changed to Enterobacterales.

Recommended action

1. Accept

Algorithm: Investigation of Nasal and Paranasal Sinus Samples

Comment number: 5

Date received: 07/05/2024
Laboratory or organisation name: UK Health Security Agency in collaboration with North Bristol NHS Trust

Under standard media workflow - list of organisms - Enterobacteriaceae should be changed to Enterobacterales.

Recommended action

1. Accept

Health benefits

Respondents were asked: 'Are you aware of any health benefits, side effects and risks that might affect the development of this UK SMI?'

Comment number: 6

Date received: 07/05/2024
Laboratory or organisation name: UK Health Security Agency in collaboration with North Bristol NHS Trust

The H&S recommendation for this SMI is for the specimen to be processed under CL2 facilities, except for risk of fungal infection with dimorphic fungi. This places significant

importance on relevant clinical details, which are often missing. Also, if the specimen is mucoid and needs to be treated with sputazol, most labs are set up with this option only being available in CL3, along with routine respiratory specimens. To prevent possible exposure and logistical issues, how about recommending that specimen processing is carried out in CL3?

Recommended action

1. Partial accept: Sentence added to the ‘Safety considerations’ section to recommend processing of specimens in CL3 in cases where clinical details regarding patients travel history are missing. However, dimorphic fungi are rare – not endemic in the UK. Therefore, it is unreasonable for all specimen processing be completed under CL3 conditions.

Financial barriers

Respondents were asked: 'Are there any potential organisational and financial barriers in applying the recommendations or conflict of interest?'.

Comment number: 7

Date received: 08/05/2024
Laboratory or organisation name: Shrewsbury and Telford Hospital NHS Trust

Increased workload and impact of this on staff. If additional staff needed to help process/read, especially mycology aspects of the investigations, a barrier would be the financial impact of staffing increased establishment as our Trust is struggling financially.

Recommended action

1. None

Improvement

Respondents were asked: Do you have any suggestions on how UK SMIs can be improved.

Comment number: 8

Date received: 07/05/2024
Laboratory or organisation name: Southwest Pathology Services

Possibly reconsider the value of having organism identification SOPs - practically all labs now have MALDI and I feel these ID SOPs are of minimal use for new or recent BMS staff (sadly!).

Recommended action

1. None: Currently, there are no plans to remove/discontinue the development of UK SMI Identification documents as some laboratories still find them useful.

Satisfaction

Respondents were asked: In general, how satisfied are you with the UK SMI service?

Comment number: 9

Date received: 07/05/2024
Laboratory or organisation name: Southwest Pathology Services

Dissatisfied. Do any of the contributors / developers work in a modern and busy clinical microbiology laboratory?

Recommended action

1. None: All UK SMIs are discussed and approved by members of the UK SMI working groups which include practising medical, clinical, and biomedical scientists representing their partner organisations. The UK SMIs also are sent out for public consultation for the wider contribution of experts and staff working in other microbiology laboratories.

   For further information, please refer to UK SMI Development.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 3			
Date received	08/05/2024	**Lab name/Professional body (delete as applicable)**	Shrewsbury and Telford Hospital NHS Trust
Health benefits			
The PCNs covered by our laboratory comprises a large rural/semi-rural population. Investigations of chronic or unusual respiratory/sinus infections would benefit our area. The risk is that we don't have an Infectious Diseases department at the hospital, so don't have the specialists to work alongside Consultant Microbiologists in the treatment of such infections, although this can likely be overcome with education and networking with local specialists.			

Recommended action 1. NONE			
Date received	21/05/2024	**Professional body**	Institute of Biomedical Science
Health benefits			
NONE			
Date received	23/05/2024	**Professional body**	Royal College of General Practitioners
Health benefits			
NONE			

Note: the document's title was 'Investigation of Nasal and Paranasal Sinus Samples' when it was released for consultation, but it has since been updated to the current document title.

UK Standards for Microbiology Investigations

Investigation of whooping cough

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Issued by the Standards Unit, National Infection Service, PHE

Bacteriology | B 6 | Issue no: 9 | Issue date: 11.05.18 | Page: 1 of 23

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 5

Scope of document 8

Introduction 8

Technical information/limitations 9

Public health management 10

1 Safety considerations 11

2 Specimen collection 11

3 Specimen transport, storage and retention 12

4 Specimen processing/procedure 12

5 Reporting procedure/interpretation 16

6 Notification to PHE, or equivalent in the devolved administrations 17

Appendix: Investigation of whooping cough 18

References 19

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	12/11.05.18
Issue number discarded	8
Insert issue number	9
Anticipated next review date*	11.05.21
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. Reorganisation of some text. Edited for clarity. Minor textual changes. All hyperlinked documents updated with the correct address. Added references to PHE guidelines where relevant.
Introduction.	Re-written for more clarity adding detailed section on molecular methods.
Technical information.	Added section for PCR and serology.
4.1 Test selection.	Developed this section to include: 4.1.1 Culture 4.1.2 PCR 4.1.3 Serology
4.5 Culture and investigation.	Added section referring to PCR and serology.
4.6 Identification.	Added section referring to identification with MALDI-TOF.
5 Reporting procedure.	Added section referring to interpreting and reporting PCR and serology results.
Appendix.	Algorithm updated to include PCR and serology as diagnostic methods with emphasis on their relevance in relation to the cough onset.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

Public Health England. (2018). Investigation of whooping cough. UK Standards for Microbiology Investigations. B 6 Issue 9. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab and blood serum.

The UK SMI describes the investigation and confirmation of *Bordetella pertussis* and *Bordetella parapertussis* by culture and PCR in pernasal swabs, nasopharyngeal aspirates and nasopharyngeal swabs and by serology in blood serum.

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

Pertussis, commonly known as whooping cough ("violent cough") has been associated with high morbidity and mortality, particularly in infants[1]. Whooping cough is a highly contagious disease that is caused by the fastidious Gram negative coccobacilli *B. pertussis* and *B. parapertussis* that colonise the respiratory tract[2]. The main symptoms include malaise, fever followed by long bursts of coughing and choking leaving the infected person gasping for breath with a characteristic whoop sound[3].

B. pertussis usually infects and causes severe respiratory disease in young children, with infants under six months of age at most risk of severe complications[3]. The infection can occur in adolescents and adults who exhibit milder symptoms of flu-like illness followed by a prolonged cough[4,5]. The incubation period of pertussis is on average between 7–10 days (range 5–21days)[2].

Despite a sustained period of high vaccine coverage, pertussis continues to display cyclical peaks in activity occurring every three to four years[6]. An increase in pertussis activity in England and Wales was observed from the third quarter of 2011, predominantly in adolescents and adults. This increase continued into 2012 and extended into infants under three months who are at highest risk of severe complications, hospitalisation and death (https://www.gov.uk/government/collections/pertussis-guidance-data-and-analysis).

In 2012, in response to a significant increase in laboratory confirmed cases of pertussis and the high rates of disease in young infants, the Health Protection Agency (Public Health England since April 2013) declared a Level 3 incident (national outbreak)[7]. On 28th September 2012, the Department of Health announced the introduction of a temporary programme to vaccinate pregnant women against pertussis[8]. This temporary programme, which is an outbreak control measure, aimed to passively protect infants from birth before they reach the age of routine immunisation and during the period of greatest risk of complications and death[9]. From the 1st April 2016, Public Health England suggests that pertussis containing vaccine should be offered to pregnant women from 16 weeks gestation, ideally after their foetal anomaly scan (usually at around 20 weeks)[10].

Diagnosis of pertussis is usually straight forward however, *formes frustes* (abortive or atypical disease; disease stopped before it has run its full course) are known to occur, and may cause diagnostic difficulty. Consideration should be given to appropriate evaluation of patients with pertussis in whom infection with *B. pertussis* or *B. parapertussis* cannot be demonstrated. In addition to sampling for pertussis, it is recommended that consideration is given to testing the patient for respiratory viruses according to local procedures.

Laboratory confirmation of clinically suspected cases can be made by culture and isolation of the causative organisms *B. pertussis* and *B. parapertussis*, detection of its DNA (typically from nasopharyngeal swabs/pernasal swabs or nasopharyngeal aspirates) or serological tests (which usually only provide a late or retrospective diagnosis)[11] (see Appendix).

Culture is conventionally performed to confirm infection with *B. pertussis* and *B. parapertussis*. The method is highly specific but sensitivity is low 20-40%. Culture is also more likely to be unsuccessful the longer the time since the onset of illness. Diagnostic sensitivity can be maximised by supplementing culture with polymerase chain reaction (PCR) methods and serology. PCR is more sensitive than culture as it does not require organisms to be viable. Serology is particularly useful in diagnosing infection in patients who have been coughing for four weeks, when both culture and PCR would be anticipated to be unhelpful[4,12-22].

Early laboratory diagnosis is important for control and prevention of whooping cough. Isolation and typing of the organism is also important for the continued monitoring of the vaccine programme. Vaccination provides the most effective strategy for preventing pertussis transmission in the population, although protection afforded by vaccination or from past infection is not lifelong[9].

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media[23-25]

The nature of selective media requires a balance between the performance characteristics and the costs of the tests. Selective media may not support the growth of all circulating strains of organisms. Refer to manufacturer's instructions and recent evidence for limitations of growth.

The media should support the growth of *B. pertussis* and *B. parapertussis*, suppress nasopharyngeal flora and be stable during storage. There are several different types of medium available that contain blood or charcoal or both, along with selective antibiotic supplements - penicillin, cefalexin or meticillin.

Meticillin is the least inhibitory of these towards *B. pertussis*, but is also the least inhibitory towards nasopharyngeal flora. Cefalexin is the most inhibitory towards nasopharyngeal flora and is superior to penicillin. For these reasons it is the antibiotic of choice for selective media in this UK SMI[25].

Primary isolation plates are incubated at 35-37°C, in an aerobic moist atmosphere maintained for 7 days[24]. A thickly poured plate is necessary to avoid desiccation on prolonged incubation.

Specimen type

Current recommendation for specimen of choice is nasopharyngeal aspirates or nasopharyngeal swabs/pernasal swabs[9]. In addition to sampling for pertussis, it is recommended that consideration is given to testing the patient for respiratory viruses according to local procedures.

Blood serum is the specimen used for pertussis serological test.

Cough plates are not recommended.

Pernasal swabs

Dacron and rayon swabs are the swabs of choice for both PCR and culture. Both types of synthetic material performed well in studies with neither superior to the other[26].

PCR and serology

It should be noted that the implementation of local PCR and serology based diagnosis of whooping cough, should be validated in the routine clinical setting before being used.

Specimen containers[27,28]

UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Public health management

Refer to the following guidance:

England and Wales:

https://www.gov.uk/government/collections/pertussis-guidance-data-and-analysis

Scotland:

http://www.hps.scot.nhs.uk/immvax/pertussis-whoopingcough.aspx

Northern Ireland:

http://www.publichealth.hscni.net/whooping-cough

1 Safety considerations[27-42]

1.1 Specimen collection, transport and storage[27-32,43]

Use aseptic technique.

Collect specimens in appropriate transport medium in CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[27-42]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[35].

As a minimum, it is recommended that the processing of any culture that may result in generation of aerosols should be processed in a microbiological safety cabinet in accordance with the relevant risk assessment, ACDP and HSE guidelines. Processing of diagnostic sample cultures that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under appropriate containment conditions as determined by risk assessment, and as required by Biological agents: managing the risks in laboratories and healthcare premises[35]. This will normally be under full CL3 conditions. Such organisms include *Mycobacterium* species, *Brucella* species, *Bacillus anthracis*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidiodes immitis,* etc.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab and/or blood, as appropriate for the test performed.

2.2 Optimal time and method of collection[44]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[44].

Swabs should be collected and transported in medium designed to support the growth of organisms.

Pernasal swabs

A pernasal swab (Dacron or rayon with flexible ultrafine wire shaft) is inserted through a nostril and advanced along the floor of the nose until it reaches the nasopharynx. It has been suggested that the swab is held against the posterior nasopharynx for up to 30s or until the patient coughs. In practice, it is more likely that a patient will only be able to tolerate this for a few seconds.

Nasopharyngeal specimens

Sampling of nasopharyngeal secretions in patients with whooping cough may precipitate a paroxysm of coughing and cause obstruction of the airways. Resuscitation equipment must be available if whooping cough is suspected. The specimen collector should avoid exposure to direct coughs from the patient.

Nasopharyngeal exudate may be obtained using a suction catheter (No.8 French) inserted through the nose. The exudate is collected in a sterile plastic trap in which the specimen is transported to the laboratory, or in a sterile clear plastic universal container (30mL or 60mL, to BS 5213).

Note: Cough plates are not recommended[45].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[46-50].

Note that culture can be affected by a number of factors, as the organism is delicate including delays in processing and specimen quality[51].

2.3 Adequate quantity and appropriate number of specimens[44]

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport, storage and retention[27,28]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[44].

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'[52].

4 Specimen processing/procedure[27,28]

4.1 Test selection

4.1.1 Culture

Laboratory confirmation is conventionally performed by culture and isolation of *B. pertussis* / *B. parapertussis* from nasopharyngeal aspirate or nasopharyngeal swab/ pernasal swab. Culture has an excellent specificity and is useful for confirming pertussis diagnosis when an outbreak is suspected.

It is best to obtain a culture from nasopharyngeal specimens collected during the first 2 weeks of cough. This is when viable bacteria are still present in the nasopharynx. After the first 2 weeks, sensitivity decreases and the risk of false-negatives increases.

It is important to note that *B. pertussis* and *B. parapertussis* are delicate organisms and therefore, processing delays may affect the likelihood of a positive culture. Sensitivity is also highly dependent on specimen quality and is affected by increasing patient age, vaccination status and length of illness.

Cultures are unlikely to be positive in adolescents and adults with more than 3 weeks of coughing[15].

It is also more difficult to recover the organism in vaccinated compared with unvaccinated children[53]. Given the limitations of culture methods, it is important to emphasise that a negative culture does not exclude pertussis.

4.1.2 PCR

PCR is usually more sensitive than culture as the organism does not need to be viable; however, PCR is less likely to be positive in patients with symptom duration of more than 4 weeks. While nasopharyngeal swabs are preferable for PCR testing, throat swabs may be used if nasopharyngeal swabs are not available, especially in community settings.

Developments in PCR have enabled the detection of co-infections and the differentiation of *B. pertussis* and *B. parapertussis* from other species of *Bordetella* [54-59].

4.1.3 Serology

Detection of anti-pertussis toxin (PT) IgG and anti-filamentous hemagglutinin (FHA) IgG antibodies in serum taken at least fourteen days after the onset of cough using an enzyme linked immunosorbent-assay (ELISA) can provide confirmatory evidence of recent infection with *Bordetella* species.

Serology may be helpful to confirm the diagnosis of whooping cough in patients with cough duration of more than 2 to 3 weeks, when culture and PCR are unlikely to yield positive results.

The anti-PT IgG serology test cannot, however, be used to determine immunity as there are currently no agreed correlates of protection. This serological assay is targeted towards older children and adults. Interpretation of anti-PT IgG levels among infants and younger children may be confounded by the presence of maternal antibodies or recent primary and booster vaccination, or show an atypical response. Data suggests that the confounding period following vaccination may be up to 10 months after the primary vaccination and up to 3 years or more after the preschool booster[60]. Therefore, serological testing should only be undertaken where there is a minimum of 1 year from primary or booster dose of pertussis containing vaccine and results should be interpreted with caution.

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

N/A

4.5 Laboratory investigation

4.5.1 Culture media, conditions and organisms

Pernasal and nasopharyngeal swabs

Inoculate each agar plate with the swab (refer to Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Nasopharyngeal aspirate

With a sterile loop select a representative portion of specimen and inoculate a loopful to each agar plate (refer to Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Pertussis or whooping cough	Pernasal swab, nasopharyngeal aspirate, nasopharyngeal swab	Charcoal blood agar with cefalexin	35-37	air, moist chamber	7d	4d and 7d	*B. pertussis* *B. parapertussis*

4.5.2 PCR and serology

Follow your local validated and approved PCR, qPCR and serology tests (ELISA or others).

The following table describes a comprehensive approach to some of the PCR targets used for detecting co-infections and for species identification and differentiation, at the time of writing [61].

Species	IS*481* [a]	*ptxS1* [b]	hIS*1001* [c]	pIS*1001* [d]
B. pertussis	+	+	-	-
B. parapertussis [e]	-	+	-	+
B. holmesii	+	-	+	-
B. pertussis and *B. paraperussis*	+	+	-	+
B. pertussis and *B. holmesii*	+	+	+	-

(a) Insertion element commonly found in *B. pertussis* and *B. holmesii*
(b) Pertussis toxin subunit S1 found in *B. pertussis* and *B. parapertussis*
(c,d) Targets present in *B. holmesii* and *B. parapertussis*, used in multiplex PCR with IS481 to detect and differentiate *B. pertussis*, *B. parapertussis* and *B. holmesii*.
(e) A specimen positive for pIS1001 may be considered to most probably contain *B. parapertussis*, but the possibility that it is positive for *B. bronchiseptica* cannot be totally excluded.

4.6 Identification

Refer to individual UK SMIs for organism identification.

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to be a rapid and powerful identification tool for cultured isolates because of its reproducibility, speed and sensitivity of analysis. The advantage

of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days[62]. Increasingly MALDI-TOF is being used to identify bacteria (including *Bordetella* species) in hospital microbiology laboratories [63]. However, there is currently very little scientific information published on use of MALDI-TOF MS for detection of *Bordetella* species[64]. Refer to UK SMI TP 40 - MALDI TOF MS test procedure.

4.6.1 Minimum level of identification in the laboratory

Bordetella species	"species" level

4.7 Antimicrobial susceptibility testing

N/A

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

For the investigation of suspected clusters or outbreaks of pertussis, please contact the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU), Colindale for the most appropriate test.

Information regarding specialist and reference laboratories is available via the following website: PHE - specialist and reference microbiology tests and services.

Refer to the PHE guidelines for public health management of pertussis.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure/interpretation

5.1 Culture

Negatives

"*Bordetella pertussis* NOT isolated" or

"*Bordetella parapertussis* NOT isolated"

Positives

"*Bordetella pertussis* isolated" or

"*Bordetella parapertussis* isolated".

5.2 PCR

Suggested reporting for the mentioned targets used in PCR detection of whooping cough.

IS*481*	***ptxS1***	**hIS*1001***	**pIS*1001***	Reporting
+	+	-	-	*B. pertussis* DNA detected
-	+	-	+	*B. parapertussis* DNA detected
+	-	+	-	*B. holmesii* DNA detected
+	+	-	+	*B. pertussis and B. parapertussis* DNA detected
+	+	+	-	*B. pertussis* and *B. holmesii* DNA detected

5.3 Serology

A case of pertussis is serologically confirmed when anti-PT IgG concentration is >70 International Units per millilitre (IU/mL) in the absence of recent vaccination (within the past year)[65].

A case of parapertussis is serologically confirmed when there is significant anti-FHA IgG increase without an increase in anti-PT IgG, IgM, and IgA antibodies. If both anti-PT IgG and anti-FHA IgG are significantly increase, the results are indicative of infection with *Bordetella* species[66].

6 Notification to PHE[67,68], or equivalent in the devolved administrations[69-72]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

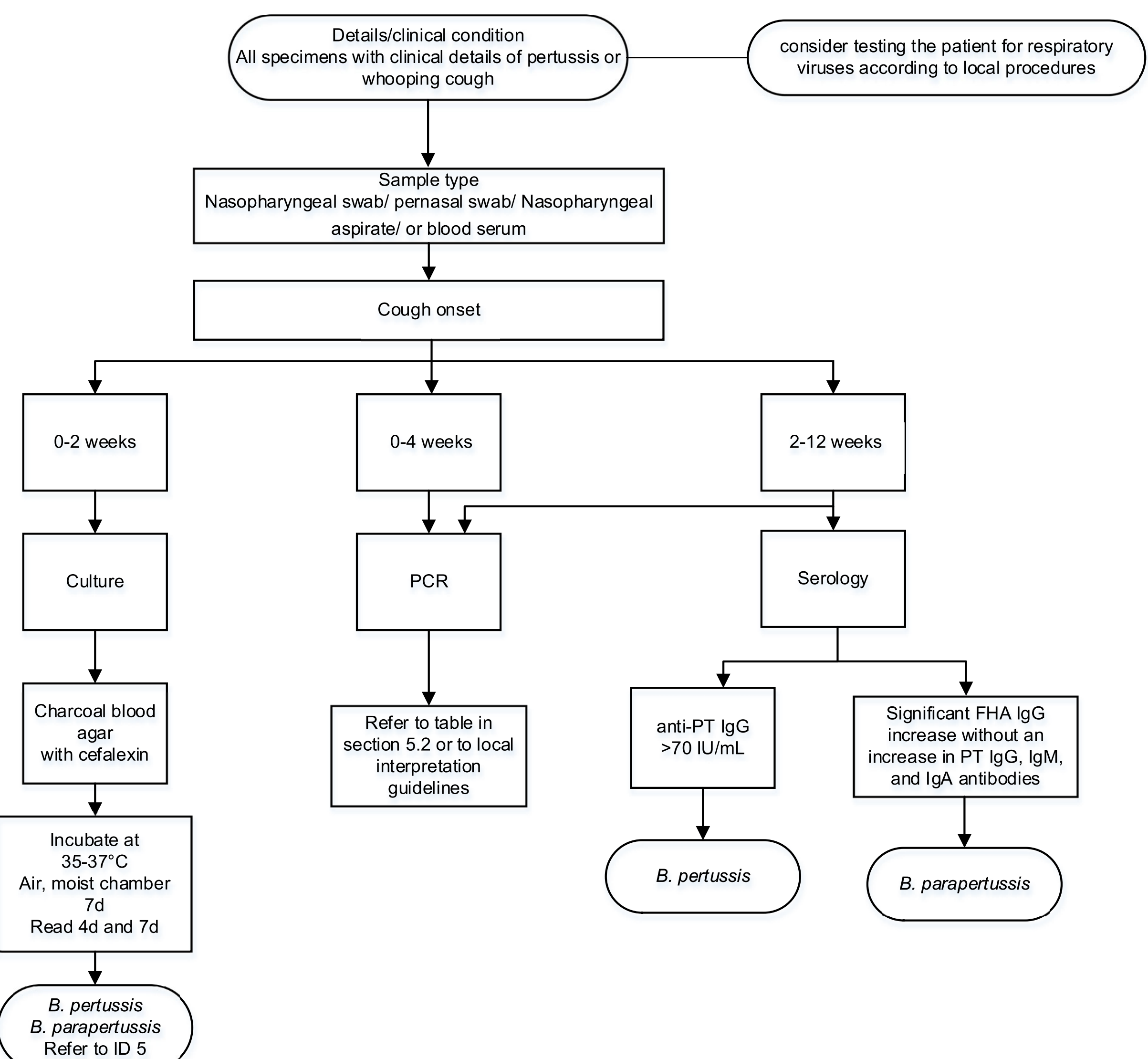
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[69,70], Wales[71] and Northern Ireland[72].

Appendix: Investigation of whooping cough



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Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

B 6 Investigation of whooping cough

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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A50711295

Consultation: 01/09/2017 – 15/09/2017

Version of document consulted on: B 6dd+

Proposal for changes

Comment number	1		
Date received	06/09/2017	**Lab name**	University Hospitals of Leicester NHS Trust
Section	5.3		
Comment			
Given that section 4.7 Antimicrobial susceptibility testing, does not give a methodology for susceptibility testing, it seems inappropriate for section 5.3 to state, Report susceptibilities as clinically indicated. Otherwise, the SMI seems to me to be a clear and useful document.			
Evidence			
Not completed.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** To add “Not applicable” in the section 4.7 of the document.		

Comment number	2		
Date received	07/09/2017	**Lab name**	PHL Bristol
Section	All of it		
Comment			
My main problem with this SMI is that is does not adequately fulfil the criteria of an SMI as stated on page 5: UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services... and UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. The document is primarily about culturing samples, although some useful information on PCR testing and a very brief mention of serological testing is also included. Since the SMI is titled Investigation of Bordetella pertussis and Bordetella parapertussis what is required is an overarching document which advises both clinician and microbiologist on the available tests, their clinical utility, and flow diagram which			

describes which tests should be used in different clinical scenarios.	
Evidence	
A joined up approach to culture based and PCR testing is lacking, leading to confusion in how best to diagnose pertussis. This apparent prevarication at SMI level is reflected by a similar disconnect in my laboratory (Bristol) and probably others. In Bristol a clinician can request B. pertussis culture on the bacteriology page of our Order Comms, B. pertussis PCR on the virology page, and B. pertussis serology on the serology page. This means that clinicians have a 2 in 3 chance of requesting the wrong test if they are not sure what test is appropriate. Given the acknowledged low sensitivity of culture, SMI should take a lead in recommending PCR as the diagnostic test of choice in a patient with recent symptom onset. The role of culture as a means of strain characterisation needs to be much more clearly defined.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	**ACCEPT** Document name changed to “Investigation of whooping cough” and rewritten in a way to make clear when to request PCR, culture or serology.

Comment number	3		
Date received	08/09/2017	**Lab name**	University Hospitals of Coventry and Warwickshire
Section			
Comment			
Page 10 - 'Meticillin' is usually spelt as 'Methicillin'.			
Evidence			
Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases 8th Edition, Volume 1 Chapter 20 pg 271.			
Financial barriers			
Not completed.			
Health benefits			
Not completed.			
Recommended	**NONE**		

action	Meticillin is correctly used.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 4			
Date received	07/09/2017	**Lab name**	University Hospitals of Leicester NHS Trust
Health benefits			
Not completed.			
Date received	08/09/2017	**Lab name**	Microbiology Antrim Area Hospital
Health benefits			
Not completed.			
Date received	15/09/2017	**Professional body**	Society for Applied Microbiology
Health benefits			
Not completed.			
Date received	15/09/2017	**Lab name**	Ninewells Hospital, Dundee
Health benefits			
None.			

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Investigation of Throat Related Specimens

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 9 | Issue no: 9 | Issue date: 15.04.15 | Page: 1 of 29

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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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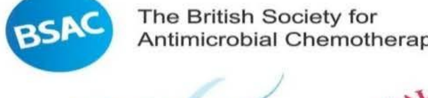

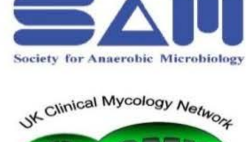

Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

SCOPE 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 14

1 SAFETY CONSIDERATIONS 16

2 SPECIMEN COLLECTION 16

3 SPECIMEN TRANSPORT AND STORAGE 17

4 SPECIMEN PROCESSING/PROCEDURE 17

5 REPORTING PROCEDURE 21

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 21

APPENDIX: INVESTIGATION OF THROAT RELATED SPECIMENS 23

REFERENCES 24

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	12/15.04.15
Issue no. discarded.	8.3
Insert Issue no.	9
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	The title of the document has been broadened to include other clinical specimens apart from throat swabs. The protocol document P 3: Recommendations for screening specimens for *Corynebacterium* species for has been merged with B 9. The whole document has been updated to reflect these changes.
Scope of Document.	This has been updated with other throat related specimens that could be requested in upper respiratory infections.
Introduction.	Laryngitis has been added as one of the several types of inflammation of the upper respiratory tract. The subheading “Rare Causes of Pharyngitis” has been updated with more information.
Safety considerations.	This section has been updated accordingly with information on Hazard Group 3 organisms.
Specimen Processing/Procedure.	Sections 2.1, 4.5, 4.6, 4.8 have been updated accordingly. All the links have been updated.
Reporting Procedure.	This section has been updated accordingly.
Appendix: Investigation of Throat Related Specimens.	The flowchart has been updated to reflect the information on the table in subheading 4.5.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested Citation for this Document

Public Health England. (2015). Investigation of Throat Related Specimens. UK Standards for Microbiology Investigations. B 9 Issue 9. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of Document

Type of Specimen

Throat swab, posterior pharyngeal swab, nasopharyngeal swab, pharyngeal washings, pus aspirate, oropharyngeal swab, throat gargle

Scope

This SMI describes the examination of bacteria and fungi from throat related specimens known to cause upper respiratory tract infections.

For more information, refer to

ID 6 – Identification of *Neisseria* species

B 51- Screening for *Neisseria meningitidis*

B 29 - Investigation of Specimens for Screening for MRSA, and

B 14 - Investigation of Abscesses and Deep-Seated Wound Infections.

For viruses that may be isolated from throat swabs, refer to G 8 – Respiratory Viruses.

This SMI should be used in conjunction with other SMIs.

Introduction

Throat related specimens are one of the most commonly performed procedures in patients with upper respiratory tract infections. This is usually carried out in primary care facilities and in emergency departments.

Upper respiratory tract infections are classified according to the type of inflammation they cause. As with many infections, the primary challenge in these conditions lies in identifying the causative pathogen and determining the extent of disease progression. There are several types of inflammation of the upper respiratory tract and they are as follows[1,2]:

- Pharyngitis (also known as sore throat)
- Tonsillitis
- Epiglottitis
- Laryngitis

Pharyngitis[1,2]

Pharyngitis is inflammation of the pharynx. It is also known as “sore throat”. This infection may be acute or chronic. Most cases are caused by viruses but it can also be caused by bacteria. Clinically, it is difficult to differentiate between bacterial and viral cause of pharyngitis based on symptoms alone. The typical symptoms are a sore throat, fever and headache but may be associated with nausea and vomiting, abdominal pain, muscle pain, scarlet fever and rashes.

Organisms commonly isolated from pharyngitis are as follows;

Streptococci

The most common cause of bacterial pharyngitis is the Lancefield group A, *Streptococcus pyogenes*. Healthy carriers of group A streptococci are usually children in whom rates of up to 20% - 30% have been reported, but rates are much lower in adults (5% - 15%)[3]. In these individuals isolation of Lancefield group A streptococci does not necessarily imply a role in infection.

Extrapharyngeal manifestations of Lancefield group A streptococcus infection can be divided into those associated with acute infection and the nonsuppurative post streptococcal sequelae such as acute rheumatic fever and glomerulonephritis, which occur 2-3 weeks after pharyngeal infection[4]. In acute infection, bacteraemia and streptococcal toxic shock may occur. Post streptococcal sequelae appear to be limited to a circumscribed set of serotypes[5].

Lancefield group C streptococci have been reported as a cause of pharyngitis[6]. The majority of the species, however, are zoonotic and rarely cause disease in humans; these include *Streptococcus equi* subspecies *zooepidemicus, Streptococcus equi* subspecies *equi* and *Streptococcus dysgalactiae* subspecies *dysgalactiae*. The beta-haemolytic group C streptococci infecting humans include the large colony form *Streptococcus dysgalactiae* subspecies *equisimilis* and the minute colony form or *Streptococcus anginosus* group (formerly the *S. milleri* group), which includes *Streptococcus constellatus* subspecies *pharyngis* and *Streptococcus anginosus*. These organisms are very rarely implicated in bacterial pharyngitis, and may express A, C, F or G Lancefield group antigens. The Lancefield group G streptococci are known to cause pharyngitis and are subdivided into the "large colony" form (which comprises the animal species *Streptococcus canis* and the human species *Streptococcus dysgalactiae* subspecies *equisimilis*, which is the only recognised causative agent of pharyngitis within the group) and the "minute colony" form (*S. anginosus*)[7].

Most of the evidence for Lancefield groups C and G streptococci causing pharyngitis comes from reports of outbreaks[8-11].

Corynebacterium diphtheriae

Diphtheria is an acute infectious disease of the upper respiratory tract and occasionally the skin. It is caused by toxigenic strains of *Corynebacterium diphtheriae* (of which there are 4 biotypes - *gravis, mitis, intermedius* and *belfanti*) and some toxigenic strains of *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*[12]. All can carry the phage-borne diphtheria toxin gene. In a fully developed case of diphtheria, this toxin damages the pharyngeal epithelium to produce a leathery membrane, giving the disease its name. This membrane may occlude the airway, sometimes causing death by respiratory obstruction. Systemic absorption by the host of the toxin from the primary site of replication may damage a wide range of cells, including those of the heart and nervous system. Myocarditis and neurological dysfunction may cause or contribute to disability or death.

The usual site of carriage or infection is the throat or nasopharynx, occasionally the nose.

Mild cases of the disease resemble streptococcal pharyngitis and the classic pseudomembrane of the pharynx may be lacking. It is thought that *C. diphtheriae* has additional virulence factors because invasive disease caused by non-toxigenic strains

has been reported[13,14]. Non-toxigenic strains of *C. diphtheriae* may be encountered in clinical specimens, especially those taken from persons previously immunised against diphtheria toxin. Non-toxigenic *C. diphtheriae* has been suggested as a cause of sore throat, but does not cause a true diphtheritic membrane or symptoms attributable to systemic absorption of toxin[12]. On re-introduction of the necessary gene, these organisms may, however, express toxin production. There is a suggestion that particular clones of non-toxigenic *C. diphtheriae* may be especially virulent as described from Russia and other former Soviet states[15,16]. Occasionally, humans will develop invasive infections with non-toxigenic strains of *C. diphtheriae*[13,14]. These conditions appear to be rare, and will be detected by blood culture rather than by culture of throat or nasopharyngeal swabs.

Although toxigenic *C. ulcerans* generally causes mild pharyngitis without any associated sequelae, at least as many cases of clinical diphtheria are now caused by *C. ulcerans* as by *C. diphtheriae* in England and Wales since the 1990s[17,18]. There is no direct evidence of person-to-person transmission of *C. ulcerans*, but it is thought that this may occur. *C. ulcerans* may infect the bovine udder and an association between human *C. ulcerans* infection and drinking raw milk has been observed[19]. However, molecular studies have indicated that domestic animals (livestock, pet cats and dogs) may be a more likely source of infection[20-22].

The pathogenic mechanism is unclear. However, as a consequence of the genome sequence being published, genes encoding adhesins, fimbriae and other products have now been identified and are thought to contribute towards pathogenicity[23].

Non-toxigenic strains in pharyngeal flora have the potential to undergo lysogenic conversion to toxin production in vivo, which may lead to disease[24].

In the 1990s there was an increase in the incidence of diphtheria in Russia and other former Soviet states, although the situation is now improving[25]. Diphtheria cases have continued to be reported from every WHO Region, especially the higher risk regions eg Africa, South East Asia and South America. Following enhancement or introduction of appropriate Public Health interventions, such as immunisation, case-finding and treatment of cases and carriers within these countries, there is now evidence that the situation has improved but there is still a strong need to maintain microbiological surveillance, laboratory expertise and an awareness of these organisms amongst public health specialists, microbiologists and clinicians[25-27].

In a susceptible population the introduction of a toxigenic strain can result in direct spread by droplet infection. Mass immunisation has resulted in the virtual disappearance of toxigenic *C. diphtheriae* from the United Kingdom, but it might not have affected the carriage of non-toxigenic strains. Most cases of toxigenic *C. diphtheriae* reported in the UK are imported from South East Asia and the Indian sub-continent and these diphtheria cases continue to be reported in South-East Asia, South America, Africa and India. A large number of UK citizens travel to and from these regions, maintaining the possibility of the reintroduction of *C. diphtheriae* into the UK[28]. However, according to the UK schedule, all travellers to epidemic or endemic areas should ensure that they are fully immunised. It also highlights the need to maintain UK routine vaccination coverage at the 95% level in the UK as recommended by the World Health Organization[17,28].

Note: For more information on the new diphtheria guidelines, see the Public Health Control and Management of Diphtheria in England and Wales publication. The

guidelines are available on the HPA legacy website diphtheria guidelines page: https://www.gov.uk/government/collections/diphtheria-guidance-data-and-analysis.

Criteria for screening throat swabs for *C. diphtheriae*

There are specific clinical associations and exposures which, if reported on requests, should trigger examination of specimens for *C. diphtheriae* or *C. ulcerans*. These are based on recognised risk factors and information from enhanced diphtheria surveillance. For more on relevant information that requests may have, see ID 2 – Identification of *Corynebacterium* species.

However, this SMI recommends testing for *Corynebacterium* species on samples from symptomatic patients for which the following information is provided:

- Membranous or pseudomembranous pharyngitis/tonsillitis
- Contact with a confirmed case within the last 10 days
- Travel abroad to high risk area within the last 10 days
- Contact with someone who has been to a high risk area within the last 10 days
- Contact with any animals (including household pets, visiting a farm or petting zoo) within the last 10 days
- Recent consumption of any type of unpasteurised milk or dairy products
- The patient works in a clinical microbiology laboratory, or similar occupation, where *Corynebacterium* species may be handled

Other causes of pharyngitis

Vincent's angina

Borrelia vincentii and *Fusobacterium* species are associated with the infection known as Vincent's angina. It is characterised by ulceration of the pharynx or gums and occurs in adults with poor mouth hygiene or serious systemic disease[29].

While there is some evidence that *Fusobacterium* species can be detected frequently from throat swabs, its prevalence in cases of acute tonsillitis has not yet been established[30,31].

Arcanobacterium haemolyticum (previously *Corynebacterium haemolyticum*)

Although *Arcanobacterium haemolyticum* is recognised as a human pathogen, this SMI does not recommend routine investigation for the organism. It has been associated with tonsillitis, pharyngitis and may cause a rash in young adults and occasionally in children[16,32]. It is suggested that in cases of treatment failure and recurrent tonsillitis, isolation of *A. haemolyticum* should be considered.

After 48hr incubation on blood agar, *A. haemolyticum* colonies exhibit narrow zones of ß-haemolysis and are approximately 0.5mm in diameter. In cases where *A. haemolyticum* is suspected, incubation of culture plates may need to be extended up to 72hr. The organism's presence may be indicated by the pitting of the agar underneath the colony; when the colony is pushed aside a minute dark pit is revealed[33].

Fungal throat and pharyngeal infections

These infections are common in patients who are immunocompromised, particularly during episodes of severe neutropenia. Patients receiving antibiotics are also prone to

fungal infections. *Candida* species may rarely cause severe invasive oesophagitis which can result in desquamation and expulsion of tissue[34]. Recognition of oropharyngeal candidosis accompanied by dysphagia indicate the possibility of oesophageal candidosis and this may be an AIDS-defining illness[35,36]. Yeast and fungal isolates from patients who are immunocompromised usually require identification and susceptibility testing.

Fusobacterium necrophorum

Fusobacterium necrophorum infection may be characterised by acute pharyngitis and fever, sometimes accompanied by membranous tonsillitis[29]. In the absence of therapy, a small number of these patients may develop the bacteraemia and metastatic infection characteristic of Lemièrre's disease, which can be life threatening[37].

Fusobacterium necrophorum has been isolated in cases of recurrent or persistent sore throat, and is a common cause of peritonsillar abscess or quinsy[38]. It is believed that up to half a million patients may present with pharyngitis due to this organism annually[39]. The literature, however, also suggests that the organism may form a minor part of the normal microflora of the upper airways in some individuals, although it has proven to be difficult to obtain primary evidence for this[37,39].

Neisseria gonorrhoeae

Pharyngeal specimens contain a variety of microorganisms including saprophytic *Neisseria* species. Identification of *Neisseria gonorrhoeae* from extragenital sites such as the oropharynx must be carefully performed and checked as a positive result can have important clinical and medico-legal implications (refer to ID 6 – Identification of *Neisseria* species). Pharyngeal colonisation may be found in patients with genital gonorrhoea, but the pharynx is rarely the only infected site[40].

Rare causes of Pharyngitis

Francisella tularensis

Oropharyngeal tularaemia (Type B tularaemia) is contracted by ingestion of contaminated food or water and it presents as stomatitis and pharyngitis. The primary ulcer is localised in the mouth, and lymph nodes of the neck region are enlarged. Physical examination shows redness and pustular changes in the mouth and pharyngeal mucous membranes, together with enlargement of regional neck lymph nodes. If tularaemia is not suspected for epidemiological reasons, the diagnosis will most likely be missed and appropriate therapy not prescribed. Identification of *Francisella tularensis* from oropharyngeal specimens should be carefully done in a microbiological safety cabinet level 2 whereas work on colonies and manipulations that might involve aerosol formation requires biological safety cabinet level 3 conditions[41].

For diagnosis, culture is more often performed for type B tularaemia in regions where this is endemic. It can be grown from pharyngeal washings, sputum specimens, and even fasting gastric aspirates in a high proportion of patients with inhalational tularaemia. It is only occasionally isolated from blood. When growth of *F. tularensis* is suspected, a reference laboratory should be consulted for safe handling and further identification.

Tularaemia occurs endemically in most countries of the Northern hemisphere, within a range of 30 to 71° latitude. The countries where the disease has been reported are Canada, the USA and Japan. Tularaemia is widely distributed over the Eurasian

continent. A high prevalence is found in the former Soviet Union and the Nordic countries, whereas the British Islands seem to be free from the disease[42].

Yersinia enterocolitica

Y. enterocolitica commonly causes enteric infections but may also infect other body sites such as lungs, bone joints, etc. Although rare, this organism has been responsible for some sporadic cases of pharyngitis[43]. This has been isolated from throats of patients with enteritis from an outbreak due to contaminated pasteurised milk[44,45]. The signs and symptoms are characterised by sore throat and fever without enteritis.

Throat swabs may be used to investigate carriage of *Yersinia enterocolitica* in patients[44].

Other uncommon organisms

Pathogens such as *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* are also uncommon causes of acute pharyngitis[3,31].

Screening for carriage in contacts

Neisseria meningitidis

Neisseria meningitidis can be spread from carrier to carrier, probably via the oral-respiratory route. A susceptible person is at risk when close contacts such as family members are identified as carriers[46].

N. meningitidis is carried on the posterior pharyngeal wall and can be detected from oropharyngeal or nasopharyngeal swabs[47]. However, posterior pharyngeal swabs seem to be better than nasopharyngeal swabs for detecting *N. meningitidis* carriage in large epidemiological studies because they identify a significantly larger number of pathogen carriers and recover a significantly larger amount of bacterial DNA[48]. Throat swabs may be an aid to diagnosis of meningococcal meningitis[49]. *N. meningitidis* can be isolated from a throat swab in about half the cases of invasive meningococcal disease (refer to B 51 - Screening for *Neisseria meningitidis*). The strain isolated from the throat is likely to be of the same group and type as that isolated from cerebrospinal fluid and blood[46]. However, other reports have described throat swabs from contacts as having no value as an aid to diagnosis because the strains from contacts are often different from those isolated from index cases[50-52].

Staphylococcus aureus

Throat swabs may be used to investigate carriage of *Staphylococcus aureus*, for example in pre-operative cardiac patients as well as to screen for carriage of Meticillin Resistant *Staphylococcus aureus* (MRSA) refer to B 29 - Investigation of Specimens for Screening for MRSA[53].

S. aureus has sporadically been reported as a cause of peritonsillar abscess. Pus may be aspirated from the abscess and sent for culture (refer to B 14 - Investigation of Abscesses and Post-Operative Wound and Deep-Seated Wound Infections).

Epiglottitis

Epiglottitis is an inflammation of the epiglottis. It commonly affects children and is associated with fever, hoarseness of voice, stridor and difficulty in swallowing. Most cases of epiglottitis in young children under the age of five used to be caused by *Haemophilus influenzae* type b but since the introduction of *H. influenzae* type b (Hib)

vaccine in October 1992, a decline in the number of cases of acute epiglottitis in children has occurred, although a minor resurgence of cases was seen in the early part of the 21st century[54]. Epiglottitis in adults is unusual and the numbers have been largely unaffected by the vaccination programme, in keeping with the more diverse range of causative organisms[55].

Capsulated *H. influenzae* type b, as well as other types should still be considered when treating epiglottitis, even in immunised children. Acute epiglottitis in young children is a rapidly progressive inflammation of the epiglottis and surrounding tissues and may result in complete airways obstruction. Because trauma from the swab may precipitate obstruction, throat swabs are contraindicated in cases of suspected acute epiglottitis. Blood cultures should be taken in all cases of suspected epiglottitis.

Treatment of *H. influenzae* type b invasive disease may not eliminate pharyngeal carriage of the organism. Failure to eradicate upper airway colonisation may impose a risk to the patient and to susceptible family contacts.

Throat swabs to determine upper airway colonisation with *H. influenzae* type b are usually only taken for epidemiological studies.

Other bacterial causes of epiglottitis include Group A beta-haemolytic streptococci, *Pseudomonas* species and *Mycobacterium tuberculosis*. *Candida* species and *Aspergillus* species are seen in immunocompromised patients.

Tonsillitis

Tonsillitis is inflammation of the tonsils, usually due to a viral infection or, less commonly, a bacterial infection. It is a common type of infection in children, although it can sometimes affect adults. Symptoms of tonsillitis include sore throat that can feel worse when swallowing, fever, coughing and headache. These symptoms will usually pass within 3-4 days.

Quinsy (peritonsillar abscess) is an acute infection located between the capsule of the palatine tonsil and the superior constrictor muscle of the pharynx[56]. Peritonsillar abscess is rare and forms, usually on one side of the throat only, with the swelling behind the tonsil near the back of the roof of the mouth. Symptoms are similar to that of tonsillitis, including dribbling, generally feeling unwell and neck swelling because of the abscess. This disease can occur in all age groups, but teenagers and young adults are most frequently affected[1]. It is usually caused by *Streptococcus* species as a complication of tonsillitis. The *Streptococcus anginosus* group (also known as *Streptococcus milleri* group) and Group A Streptococci have been established as key organisms in peritonsillar abscess[57].

Fusobacterium necrophorum and *Fusobacterium nucleatum* are also comparatively common causes of quinsy[38,57]. Anaerobic organisms predominantly isolated in peritonsillar abscesses include *Prevotella, Porphyromonas* and *Peptostreptococcus* species[58,59].

S. aureus has sporadically been reported as a cause of quinsy. Pus may be aspirated from the abscess and sent for culture (refer to B 14 - Investigation of Abscesses and Deep-Seated Wound Infections).

Arcanobacterium haemolyticum has been associated with tonsillitis, pharyngitis and may cause a rash in young adults and occasionally in children[16,32]. It is suggested that in cases of treatment failure and recurrent tonsillitis, isolation of *A. haemolyticum* should be considered.

Laryngitis

Laryngitis is inflammation of the larynx (voice box). In most cases, laryngitis is caused by a viral infection (such as a cold), or voice strain or by bacteria such as *Corynebacterium diphtheriae*, although this is rare[3]. There is also a recent case report that suggests that MRSA has been implicated in laryngitis[60]. This eases without treatment within a week. This is known as acute laryngitis. Symptoms of laryngitis include hoarseness, loss of voice and sore throat.

Laryngitis can occasionally have other causes, such as smoking, alcohol misuse, voice overuse, reflux of acid from the stomach (also called gastroesophageal reflux disease (GERD)), rare infections or allergies, or inhalation or irritants or chemicals[61]. The symptoms do last much longer. This is known as chronic laryngitis.

Other less common causes of chronic laryngitis are bacterial (Group A streptococci, *Streptococcus pneumoniae, Haemophilus influenzae* and *Mycobacterium tuberculosis*) or fungal (*Candida* species, *Blastomyces* species) infections and parasite infections.

For viruses that may be isolated from throat swabs, refer to G 8 – Respiratory Viruses.

Screening of neonates

Surveillance screening of neonates may include a throat swab.

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[62,63]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Incubation

The duration of incubation can affect the throat culture result. Once plated, a culture should be incubated at 35°C–37°C for 18–24 hours before reading. Additional incubation overnight at room temperature may identify a number of additional positive throat culture results. However, although initial therapeutic decisions may be made on the basis of overnight culture, it is advisable to re-examine plates at 48 hours that yield negative results at 24 hours[64].

1 Safety Considerations[62,63,65-79]

1.1 Specimen Collection, Transport and Storage[62,63,65-68]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[62,63,65-79]

Hazard Group 2 Organisms

C. diphtheriae and *C. ulcerans* are in Hazard group 2; suspected and known isolates of *C. diphtheriae* /*C. ulcerans* should always be handled in a microbiological safety cabinet. Sometimes the nature of the work may dictate that full containment Level 3 conditions should be used eg for the propagation of *C. diphtheriae/C. ulcerans* in order to comply with COSHH 2004 Schedule 3 (4e). For the urease test a urea slope is considered safer than a liquid medium.

N. gonorrhoeae and *N. meningitidis* are also Hazard group 2 organisms. Although for *N. meningitidis*, the processing of diagnostic samples can be carried out in a microbiological safety cabinet at Containment Level 2 but due to the severity of the disease and the risks associated with generating aerosols, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet in a containment level 3 facility until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols)[71].

Haemophilus influenzae is a Hazard Group 2 organism, and, and in some cases the nature of the work may dictate full Containment Level 3 conditions[80].

Hazard Group 3 Organisms

F. tularensis is a Hazard group 3 organism, one of the most potent pathogens known in human medicine and evokes great concern as a bioterrorism agent[81].

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[71].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

Throat swab, posterior pharyngeal swab, nasopharyngeal swab, pharyngeal washings, pus aspirate, oropharyngeal swab, throat gargle

2.2 Optimal Time and Method of Collection[82]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[82].

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[83-87].

Throat swabs should be taken from the tonsillar area and/or posterior pharynx, avoiding the tongue and uvula.

Throat culture should not be taken if the epiglottis is inflamed as sampling may cause serious respiratory obstruction.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate Quantity and Appropriate Number of Specimens[82]

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage[62,63]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[82].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[82].

Ideally, inoculation of specimens for *N. gonorrhoeae* should be made directly on to culture media at the time of collection and these should be incubated without delay. Transport time should be as short as possible[47].

4 Specimen Processing/Procedure[62,63]

4.1 Test Selection

N/A

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Stain for Vincent's organism if clinically indicated (refer to TP 39 – Staining Procedures).

4.4.2 Supplementary / Preparation of smears

N/A

4.5 Culture and Investigation

Inoculate each agar plate with swab (refer to Q 5 – Inoculation of Culture Media in Bacteriology).

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)‡
			Temp °C	Atmos	Time		
Pharyngitis (Sore throat) Epiglottitis Tonsillitis Laryngitis	Throat swab	Blood agar[7]* OR	35-37	Anaerobic	18-24hr	≥18hr	Lancefield group A, C and G streptococci
		Staph/Strep selective agar** [88,89]	35–37	Aerobic	18-48hr	≥24hr	
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Membrane formation or membranous pharyngitis/tonsillitis Foreign travel to high risk area	Throat swab **OR** Nasopharyngeal swab	Hoyle's tellurite agar	35-37	Air	18-48hr	daily	*C. diphtheriae* and *C. ulcerans*
S. aureus (MSSA) carriage	Throat swab	Blood agar** Chromogenic agar[90]	35-37	5-10% CO_2	18-24hr	≥18hr	*S. aureus*
GUM clinic, gonorrhoea, *N. meningitidis* case or contact	posterior pharyngeal swab **OR** Nasopharyngeal swab	GC selective agar	35-37	5-10% CO_2	40-48hr	≥40hr	*N. gonorrhoeae* *N. meningitidis*
Treatment failure and recurrent tonsillitis	Throat swab	Blood agar	35-37	5-10% CO_2	40-48hr***	≥48hr	*A. haemolyticum*[33]
Epiglottitis	Throat swab	Chocolate agar	35-37	5-10% CO_2	24 - 48hr	daily	*H. influenzae*
Diabetes, Immunosuppressed, Oral candidosis	Throat swab **OR** Oropharyngeal swab	Sabouraud agar **OR** Chromogenic agar# [91-93]	35-37	Air	40-48hr	≥40hr	Yeasts Mould
Clinical	**Specimen**	**Optional**	**Incubation**			**Cultures**	**Target**

details/ conditions		media	Temp °C	Atmos	Time	read	organism(s)
Persistent sore throat or Quinsy	Pus aspirate **OR** Throat swab	Fastidious Anaerobic Agar (FAA) containing nalidixic acid and vancomycin	35-37	Anaerobic	5-7 d	≥48hr	*F. necrophorum*[38]
Other organisms for consideration – MRSA (B 29 - Investigation of Specimens for Screening for MRSA). *Francisella tularensis* and *Yersinia enterocolitica* although uncommon causes of throat related infections, may be considered. *F. tularensis* has been isolated from oropharyngeal specimens, pharyngeal washings, sputum specimens, and even fasting gastric aspirates. It has also only occasionally been isolated from blood[42]. Other predominant anaerobic organisms isolated in peritonsillar abscesses are *Prevotella, Porphyromonas* and *Peptostreptococcus* species[58,59].							
*Alternatively, the blood agar could also be incubated in 5-10% CO_2 at 35-37°C for 18 – 24hr[6,64] **Staphylococcus/Streptococcus* selective agar may be used for Lancefield group streptococci and staphylococci. The duration of incubation can affect throat culture result and so for increased isolation rate of Lancefield group A streptococci, further incubation of culture plates for 40-48hr is done and then re-examined[64]. ***May be extended to 72hr. # There is a wide range of commercially available chromogenic culture media for the isolation of yeasts. Manufacturer's instructions on use must be followed[91,92]. ‡ For appearance of relevant target organism see individual SMIs for organism identification.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

C. diphtheriae	species level; urgent toxigenicity test / refer to Ref Lab
C. ulcerans	species level; urgent toxigenicity test / refer to Ref Lab
H. influenzae	species level; type b or not if epiglottitis, refer to Ref Lab
β haemolytic streptococci	Lancefield group level
A. haemolyticum	species level
N. gonorrhoeae	species level
N. meningitidis	species level
S. aureus	species level
Yeasts	"yeasts" level *
Fusobacterium species	species level
* Yeast and fungal isolates from patients who are immunocompromised usually require identification and susceptibility testing.	

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: All work on suspected isolates of *C. diphtheriae* which is likely to generate aerosols must be performed in a safety cabinet.

A medical microbiologist must be informed of all suspected isolates of *C. diphtheriae* as soon as possible, so that a risk assessment can be undertaken for rapid referral for toxin testing. Toxigenicity testing is available and undertaken only by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) PHE Colindale.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

4.8 Referral for Outbreak Investigations

Diphtheria

As diphtheria is a notifiable disease in the UK, for public health management of cases, contacts and outbreaks, all suspected clinical cases should be notified immediately to the local Public Health England Centres.

Isolates of *C. diphtheriae* for which information available is suggestive of clinical diagnosis of diphtheria should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures and all such isolates should be referred to the national reference laboratory for toxigenicity testing.

Group A Streptococci (GAS) infection[94]

Clinicians, microbiologists and health protection teams (HPTs) should be mindful of potential increases in invasive disease and maintain a high index of suspicion in relevant patients as early recognition and prompt initiation of specific and supportive therapy can be lifesaving. Invasive disease isolates and those from suspected clusters or outbreaks should be submitted immediately to the Respiratory and Vaccine Preventable Bacteria Reference Unit at Public Health England, 61 Colindale Avenue, London NW9 5EQ. For more information, refer to https://www.gov.uk/streptococcal-infections.

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

Stain for Vincent's organisms: report on Vincent's organisms detected.

5.1.1 Microscopy reporting time

Report results for Vincent's organisms as soon as available within 24hr of receipt.

5.2 Culture

Negatives

"β-haemolytic streptococci of Lancefield group A, C and G not isolated".

"*Corynebacterium diphtheriae* not isolated".

Also, report results of supplementary investigations.

Positives

Report clinically significant organisms isolated.

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically stating, if appropriate, that a further report will be issued.

Written report, 16–72hr.

Supplementary investigations eg toxigenicity testing of *C. diphtheriae* to be issued when available.

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[95,96] or Equivalent in the Devolved Administrations[18,97-100]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many

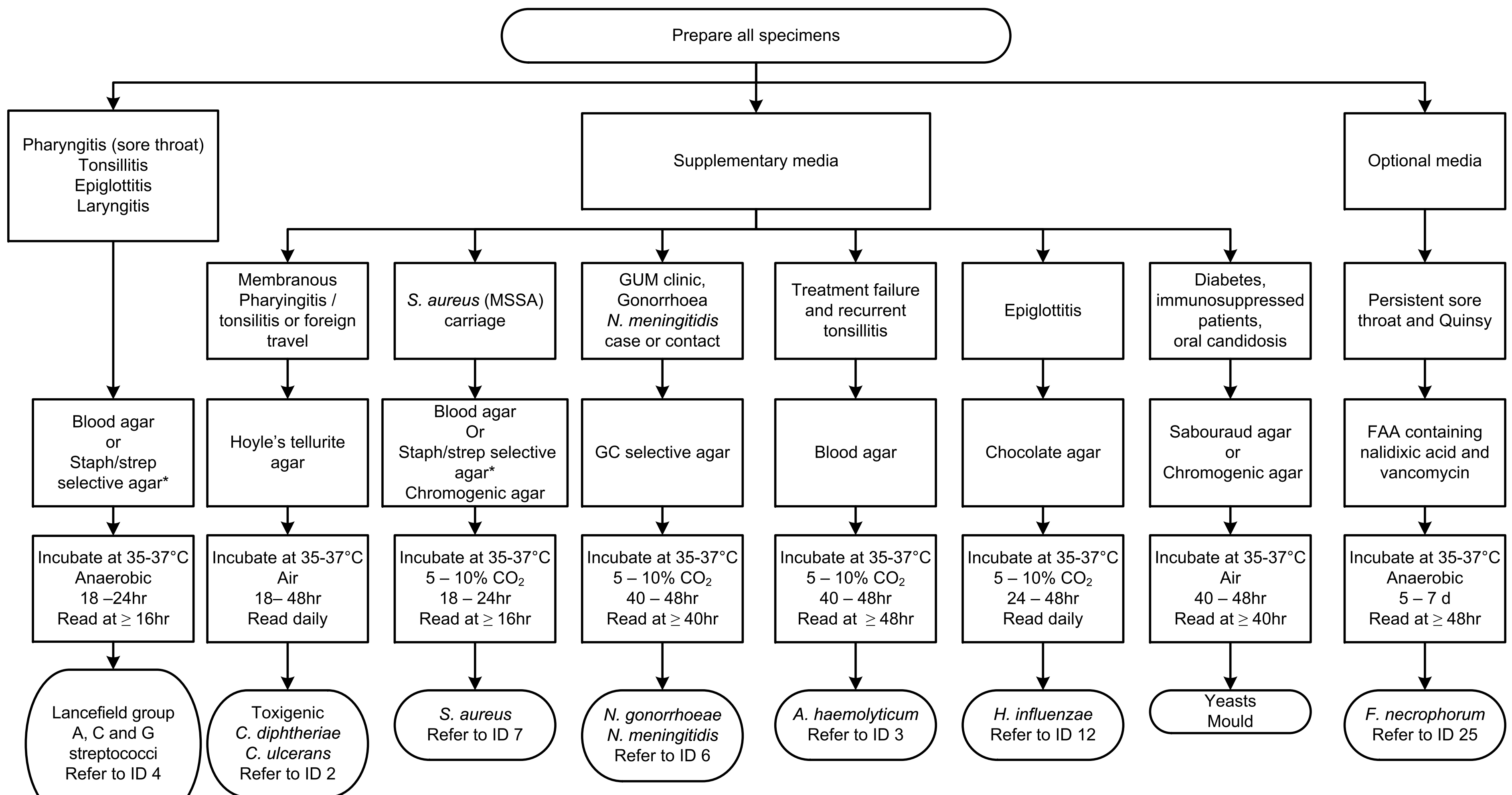
PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[97,98], Wales[99] and Northern Ireland[100].

Appendix: Investigation of Throat Related Specimens



*Staphylococcus/Streptococcus selective agars may be used for Lancefield group streptococci

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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 9 Investigation of Throat Related Specimens

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation 14.05.14 – 02.06.14

Version of document consulted on – B 9dn+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	15/05/2014	**Lab Name**	York Hospital
Section	4.5.1		
Comment			
a. Pharyngitis appears in both sections: Routine: Blood agar anaerobic incubation Add: Blood agar for *A. haemolyticum*. Could we get clarification please? b. By looking at the culture table, is it better to say for all swabs blood agar - incubate anaerobically for haemolytic strep (Table first section saying - all swabs Second section leaving as it is). My reason is pharyngitis and tonsillitis appears on both sections and creates confusion as to which section to follow.			
Financial Barriers			
No.			
Health Benefits			
No.			
Recommended Action	a. **NONE** The table in section 4.5.1 explains itself, the first section explains all standard media that should be plated under the clinical conditions mentioned as well as their incubation and the second section shows all supplementary media that should be plated in these conditions. The standard media for pharyngitis is blood agar incubated anaerobically at 35-37°C for 16-24hr and the supplementary media needed are Hoyle's tellurite agar incubated at 35-37°C in air for 16-48hr and blood agar incubated at 35-37°C in 5-10% CO_2 for 40-48hr. b. **NONE** Looking at the type of specimens mentioned under the scope of this document, it cannot be narrowed down only to all swabs as some specimens may be pharyngeal washings, pus aspirate or mouth gargle.		

Comment Number	2		
Date Received	27/05/2014	**Lab Name**	Golden Jubilee National Hospital

Section	Introduction page 10
Comment	
Sense check required for first sentence under *Fusobacterium necrophorum*.	
Evidence	
see p10 of draft B9 document.	
Financial Barriers	
No.	
Health Benefits	
No.	
Recommended Action	**ACCEPT** This sentence has been corrected to read correctly.

Comment Number	3		
Date Received	30/05/2014	**Lab Name**	Truro, Cornwall
Section	4.5.1		
Comment			
Page 19 - 4.5.1 Clinical details column, 5-7 days, how do you define persistent sore throat or Quinsy when testing for *F. necrophorum*.			
Recommended Action	**NONE** This was discussed and it was agreed by the Working Group that it should be left as it is and not changed.		

Comment Number	4		
Date Received	01/06/2014	**Professional Body**	Healthcare Infection Society
Section	Other causes of pharyngitis page 10 *Fusobacterium necrophorum* section		
Comment			
The first sentence of this paragraph appears to include the extra word 'antibiotic' or else some words are missing from the sentence.			
Financial Barriers			
No.			
Health Benefits			

No.	
Recommended Action	**ACCEPT** This sentence has been corrected to read correctly.

Comment Number	5		
Date Received	02/06/2014	**Professional Body**	UK CMN
Section	4.6.1 & 6		
Comment			
a. Section 4.6.1 Yeast level Footnote needed regarding ID in immunocompromised individuals (there is an earlier statement in the text). b. Section 6 New PHE guidelines on diphtheria just published: 'Public health control and management of diphtheria (in England and Wales). Interim guidelines. Diphtheria Guidelines Working Group, PHE, London, 2014.'			
Financial Barriers			
No.			
Health Benefits			
No.			
Recommended Action	a. **ACCEPT** The footnote regarding identification in immunocompromised individuals has been added. The statement has been phrased as "*Yeast and fungal isolates from patients who are immunocompromised usually require identification and susceptibility testing*". b. **ACCEPT** This new PHE guidelines on diphtheria has been added to section 6.		

2nd Consultation 18.08.14 – 22.09.14

Version of document consulted on – B 9 and P 3 merged di+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	20/08/2014	**Lab Name**	Microbiology Aberystwyth
Section	Pharyngitis / Tonsillitis, Table 5.4.1 - *Fusobacterium necrophorum*		
Comment			

<table>
<tr><td colspan="2">This section sounds a little dated now, and the word 'acute' could certainly be added to recurrent and persistent. Evidence suggests that diagnosing and treating *F. necrophorum* throat infections early (ie primary infection) will prevent the development of more serious disease, eg Lemierre's or peritonsillar abscess, as well as preventing recurrent or persistent infection (which the vast majority of F. necrophorum infections go on to become). In our own (as yet unpublished) studies, we achieved an isolation rate of 15-16% in all throat swabs - higher even than Group A Strep - and the commonest clinical information given was 'acute tonsillitis', with the second highest 'recurrent tonsillitis'.

Mention should be made of the commonest age range of patients with *F. necrophorum* (adolescents / young adults). In Viborg, Denmark (the leading centre for research into *F. necrophorum*), all patients between the age of 10 and 40 are screened.
F. necrophorum has now been identified in asymptomatic patients using PCR, but the evidence suggests that it is carried in far lower numbers than in symptomatic patients - also, culture of F. necrophorum is very rare in asymptomatic individuals. Re Table 5.4.1, only the word 'persistent' is used but not 'recurrent' for circumstances under with we should look for *F. necrophorum*. Our All Wales SOP was unfortunately based on this table and not the accompanying background info. Luckily, my lab manager has added the 'recurrent' to our local Throat Swab SOP, but this is not the case for most labs in Wales. In my opinion (and most others who have worked in this field) not culturing in patients with recurrent sore throats will result in massive under-detection of *F. necrophorum* - ie large numbers of symptomatic patients being incorrectly told that their swabs are negative. I realise that there is a cost burden to laboratories, and also training is an issue (happy to help!), but are we not duty bound to come up with the right answer?!</td></tr>
<tr><td colspan="2">Evidence</td></tr>
<tr><td colspan="2">I sent these comments quickly (and off the top of my head!) as I didn't want to miss the deadline. I have quite a few references, and there is plenty of ongoing research in this field. Most agree though that we need to take *F. necrophorum* much more seriously. Discussion with the Anaerobe Reference Lab in Cardiff would also be beneficial.</td></tr>
<tr><td colspan="2">Financial Barriers</td></tr>
<tr><td colspan="2">Yes.</td></tr>
<tr><td colspan="2">Health Benefits</td></tr>
<tr><td colspan="2">If the criteria for culture of *F. necrophorum* were widened, then a significant number of patients suffering from (often debilitating) acute / recurrent / persistent sore throats would be correctly diagnosed by microbiology labs, leading to more effective antibiotics being issued where necessary (another area that needs to be looked at). Furthermore, this could prevent a small proportion of these patients from developing serious illness, eg peritonsillar abscess or Lemierre's disease. Overall, this could provide a cost benefit to the country, as a large number of work days are lost to sore throats annually, and fewer visits to GPs could result if recurrent / persistent infections are effectively treated. Furthermore, I believe that *F. necrophorum* plays an important role in the pathway to tonsillectomy - but more research is required in this area.</td></tr>
<tr><td>Recommended Action</td><td>PARTIAL ACCEPT

This was discussed with the Working Group members and it was agreed that *F. necrophorum* is still not considered as</td></tr>
</table>

	significant when screening throat swabs routinely. Relevant information has been included in the introduction.

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 6			
Date Received	15/05/2014	**Lab Name**	Southampton City Clinical Commissioning Group
Date Received	15/05/2014	**Lab Name**	Nottingham NUH
Date Received	19/05/2014	**Lab Name**	Royal Oldham Hospital
Date Received	31/05/2014	**Lab Name**	Microbiology
Date Received	21/08/2014	**Lab Name**	Public Health wales
Date Received	19/09/2014	**Lab Name**	Truro Microbiology

UK Standards for Microbiology Investigations

Processing of Faeces for *Clostridium difficile*

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 10 | Issue no: 1.7 | Issue date: 19.09.18 | Page: 1 of 24

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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 6

SCOPE OF DOCUMENT 8

SCOPE 8

INTRODUCTION 8

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 11

2 SPECIMEN COLLECTION 11

3 SPECIMEN TRANSPORT AND STORAGE 12

4 SPECIMEN PROCESSING/PROCEDURE 12

5 REPORTING PROCEDURE 18

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 19

REFERENCES 20

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	7/19.09.18
Issue no. discarded.	1.6
Insert Issue no.	1.7
Section(s) involved	**Amendment**
Section 4.8 Referral for Outbreak Investigations	This section has been updated to cover the arrangements within Northern Ireland for referring outbreak investigations.
Whole document.	Scientific content remains unchanged.

Amendment No/Date.	6/03.09.18
Issue no. discarded.	1.5
Insert Issue no.	1.6
Section(s) involved	**Amendment**
Specimen processing/procedure.	In section 4. 3.1, the alcohol shock method has been clarified and made easier to understand for users. The timing for incubation has been updated from 30min to 60min and references added to support this. Section 4.6 updated with information on the minimum level of identification in the laboratory. Some information formerly in section 4. 5 moved up to section 4.3.1 as it reads better.
Whole document.	Scientific content remains unchanged.

Amendment No/Date.	5/02.04.14
Issue no. discarded.	1.4
Insert Issue no.	1.5

UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
A50711295

Section(s) involved	Amendment
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	4/29.03.12
Issue no. discarded.	1.3
Insert Issue no.	1.4
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC[1,2]. Edited for clarity. Reorganisation of some text. Minor textual changes.
Technical Information / Limitations.	Text to describe the use of the term CE marked leak proof container added.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
Notification to HPA.	Standard text added.
References.	Some references and hyperlinks updated.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives
http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2018). Processing of Faeces for Clostridium difficile. UK Standards for Microbiology Investigations. B 10 Issue 1.7.
http://www.hpa.org.uk/SMI/pdf

Scope of Document

Type of Specimen

Faeces

Scope

This SMI describes the culture and identification of *Clostridium difficile* from faeces. It advises that all samples selected for investigation should be tested in the first instance, with a toxin detection kit or cell cytoxin assay. The laboratory can then either store toxin-positive faeces for culture at a later time if required or, if from a laboratory in England, they can request access to the *C. difficile* Ribotyping Network for England (CDRNE) through the Regional Microbiologists. Isolates may be referred to the Anaerobe Reference Laboratory from elsewhere in the UK (except Scotland) for outbreak investigation, in conjunction with the Regional PHE laboratories under the DH/PHE surveillance scheme, or to the PHE Centre for Infection (Laboratory of HealthCare Associated Infection). In Scotland isolates may be submitted to the Scottish *C. difficile* Reference Service according to criteria developed in conjunction with Health Protection Scotland (HPS) as part of the mandatory surveillance programme:

http://www.documents.hps.scot.nhs.uk/hai/sshaip/guidelines/clostridium-difficile/protocol-scottish-surveillance-programme-cdad-2009-03.pdf

B 30 - Investigation of Faeces Specimens for Enteric Pathogens and ID 8 - Identification of *Clostridium* Species are recommended for additional background information.

Introduction

Clostridium difficile Infection (CDI) and Antibiotic Associated Diarrhoea (AAD)

C. difficile is a Gram positive, spore forming, strictly anaerobic rod, so named because of the difficulty in original culture and characterisation[3]. Toxigenic strains produce large protein toxins A and B, both being major virulence factors[4]. Most disease associated with *C. difficile* is intestinal, though *C. difficile* may be isolated from blood or tissues[5-9].

Changes in the gut flora associated with broad spectrum antibiotics and chemotherapeutic agents can result in colonisation by *C. difficile*; it is the commonest identifiable cause of AAD[10]. Almost all drugs with an antibacterial spectrum of activity have been implicated causally in AAD. The most frequently implicated drugs are those which have a marked effect on the microflora of the colon. These include broad spectrum beta lactams, cephalosporins, clindamycin and fluoroquinolones[11]. The incidence of *C. difficile* infection has been shown to decrease once antibiotic therapy is controlled.

The production of two toxins A (enterotoxin) and B (cytotoxin) causes the characteristic mucosal damage consisting of plaque-like lesions leading to the formation of a pseudomembrane. Not all strains of *C. difficile* produce toxin, and therefore not all can cause illness.

The spectrum of disease ranges from a self-limiting mild diarrhoea, to the advanced and severe illness characteristic of pseudomembranous colitis. The most accurate diagnosis of pseudomembranous colitis is affected by endoscopic detection of colonic pseudomembranes or microabscesses in antibiotic-treated patients who are suffering from diarrhoea, and who have *C. difficile* and its toxins in their stools.

The organism has been associated with outbreaks in hospitals and in extended care facilities for the elderly[12]. It represents an important cause of hospital-acquired infection. *C. difficile* can be isolated from soil, hospital environments and both human and animal faeces[13]. It is rarely found in the flora of normal adults, but up to 50% of infants may be colonised in the first few months, although disease is rarely present at this age[14,15]. *C. difficile* infection is more common in the elderly. The reasons for this are not clear, although there is some evidence to suggest that these patients have a less effective natural barrier to infection[16]. The importance of age can be demonstrated by figures from CDSC which show that 81% of cases (in which age was reported) were from patients >65 years old[17].

Elderly medical patients, those undergoing general surgery, oncology patients and those with chronic renal disease are at particular risk of infection by *C. difficile*[18-21].

Clostridium difficile Toxins and Toxin Detection

Demonstration of *C. difficile* toxins in diarrhoeal stools is generally regarded as being suggestive of CDI, in the absence of any other recognised cause for gastrointestinal disturbance. In outbreaks it is suggested that primary culture of the organism is undertaken in tandem with toxin detection[22]. The culture of toxin negative faeces followed by toxin testing of the isolate may increase the number of patients diagnosed[23].

Although considered by some to be the ‘gold standard’, use of tissue culture for the detection of *C. difficile* toxins by virtue of its cytopathic effect (neutralisable with *C. sordellii* antitoxin) requires technical expertise, and involves usually a 24 (up to 48) hour delay for the final result[24]. Tissue culture, especially with Vero cells, may detect other faecal cytotoxins that are associated with diarrhoea, eg, *C. perfringens* enterotoxin[22]. Cytopathic effect (CPE) that is not neutralised by *C. sordellii* antitoxin may indicate that another pathogen is present.

There are numerous commercially available EIA tests intended to detect the toxins of *C. difficile*. Some detect Toxin A, others A and B, although the sensitivity and specificity of these are variable[25-29]. Commercial EIAs that detect both toxins A and B are considered more appropriate than those which detect A alone, because infection due to A- B+ strains has been recorded[30].

Latex agglutination kits are available, but are not as accurate as EIA due to poor sensitivity[31]. Detection by counter immuno-electrophoresis (CIE) has been suggested, but this method lacks sensitivity and specificity, and is not recommended[32,33].

These and other testing procedures are reviewed in a recent report presented to the Department of Health[34].

Typing of *C. difficile*

Typing of isolates of *C. difficile* is sometimes useful in the investigation of multiple cases of infection. Typing methods that have been used include bacteriophage/bacteriocin typing and serotyping[35,36]. PCR ribotyping is gaining

acceptance as an internationally recognised method and within England a PCR ribotyping network (CDRNE) has been established by the PHE for use when there is an increase in frequency of CDI, or increased severity, complication, recurrence or death rate associated with CDI[37]. The Anaerobe Reference Laboratory in Cardiff currently provides the same service for Wales and the rest of the UK (except Scotland) and performs the typing for the DH/PHE surveillance scheme In England. In Scotland this service is provided by the Scottish *C. difficile* Reference Service, which is based at the Scottish *Salmonella* Reference Laboratory in Glasgow. Ribotyping and other more refined, molecular methods of strain differentiation are performed at the Centre for Infection. Other methods include cell surface protein profiles and other DNA-based methods of analysis[38-40].

Other Organisms Associated with AAD

In addition to *C. difficile*, infection with *C. perfringens*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Candida* species and *Salmonella* species have been implicated with AAD[41,42].

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen Containers[1,2]

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.

1 Safety Considerations[1,2,43-57]

1.1 Specimen Collection, Transport and Storage[1,2,43-46]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[1,2,43-57]

Containment Level 2.

Containment Level 3 if the following organisms are suspected from clinical information or laboratory findings:

- *Salmonella* Typhi
- *Salmonella* Paratyphi A, B and C
- Vero cytotoxin producing *E. coli* O157 (VTEC)
- *Shigella dysenteriae*

Under normal circumstances, a culture for *C. difficile* would not be requested from patients suspected of having any of the above organisms. However, if clinical details or routine culture indicate any of the above, then all specimen preparation and culture for *C. difficile* should be performed in the cabinet in Containment Level 3.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[49].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

Faeces

2.2 Optimal Time and Method of Collection[58]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[58].

Specimen may be passed into a clean, dry, disposable bedpan or similar container, and transferred into a CE marked leak proof container. The specimen is unsatisfactory if any residual soap, detergent or disinfectant remains in the pan.

Formed stools are unsuitable for investigation for *C. difficile*. These should be rejected with the appropriate comment appended to the report.

2.3 Adequate Quantity and Appropriate Number of Specimens[58]

A liquid specimen of 1-2ml is sufficient for culture and toxin detection. Repeat testing of samples if there is no indication within a 28 day period. This applies to repeat testing of positive results. On the contrary, a negative test, if symptoms persist, should be re-tested as it is known that a one-off negative can occur.

In suspected outbreaks, samples should be stored at 4°C or frozen at -20°C for culture.

Isolates confirmed as C. difficile may be referred for ribotyping in accordance with the Anaerobe Reference Laboratory (ARL: guidelines for typing investigations listed on-line at: www.hpa.org.uk/cfi/arl) or the relevant Scottish guidance:

http://www.documents.hps.scot.nhs.uk/hai/sshaip/guidelines/clostridium-difficile/smf-recommended-protocol-testing-for-cdiff-2009-12.pdf

In general sending 10 isolates from each outbreak should be sufficient.

3 Specimen Transport and Storage[1,2]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[58].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[44]. Refrigerate for up to two days if unable to process within two hours. Freeze at -20°C or below if unable to process within two days of collection[59].

All *C. difficile* toxin-positive faecal samples should be kept refrigerated, or frozen, so that culture can be performed to recover isolates for typing[60]. It is not necessary to keep the whole specimen; an aliquot in a small eppendorf would suffice. The duration of storage needs to be determined locally, but should allow appropriate outbreak investigation.

4 Specimen Processing/Procedure[1,2]

4.1 Test Selection

If clinically indicated, patients who fulfil any of the following criteria should be screened: antibiotic-associated diarrhoea (everyone over the age of two); pseudomembranous colitis; and post-antibiotic treatment on all patients over 65 years old (in Scotland, all patients over 65 years old with diarrhoea)[61].

Manufacturers' instructions must be followed when using toxin detection kits. It is recommended that a kit that is capable of detecting both A and B toxins is used. In order to ensure that strain type monitoring can be carried out in the event of an outbreak, samples of all positive stool samples should be at 4°C or -20°C for later culture[60].

The culture and identification of *C. difficile* from faeces is intended to be followed in outbreaks, or as part of enhanced surveillance. This method is described in section 4.5 Culture and Investigation.

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.3.1 Standard

Alcohol shock method[62-64]

The advantage of using alcohol shock for selection of *C. difficile* is that only spores should survive this process, and it eliminates the growth of other non-sporing faecal organisms. The selective agents are usually based on cefoxitin and cycloserine (although others have been described), and these are usually inhibitory to most other clostridial species. The resulting growth from an active case of infection is often a pure culture of *C. difficile*.

It should be noted that the same medium from different suppliers may give different colonial appearances and the descriptions given here are not absolute.

- Make a suspension of approximate equal parts faeces and absolute alcohol (that is for example, 1g of stool sample added to 1ml of absolute alcohol. If stool sample is liquid, the same applies too.) in a screw capped glass bijou.
- Vortex gently and leave at room temperature for 60min.
- With a disposable pastette, inoculate two drops (approx. 50 -75 µl) of the deposit to cefoxitin-cycloserine egg yolk agar* (CCEY) selective agar and streak for single colonies. At the same time, inoculate the control organisms on CCEY from their spore suspension and incubate as outlined below
- Incubate anaerobically at 35°C - 37°C for 48 - 72hr.

Note: Cultures may be examined after overnight incubation, but should not be removed from the anaerobic cabinet because sporulation is inhibited on selective media and young cultures may die on exposure to air. If using anaerobic jars, cultures must not be examined before 48 hours incubation

* Egg-yolk supplement is optional; blood agar can also be used.

4.3.2 Supplementary

N/A

4.4 Microscopy

4.4.1 Standard

N/A

4.4.2 Supplementary

N/A

4.5 Culture and Investigation

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
As clinically indicated on patients who fulfil the following criteria: antibiotic-associated diarrhoea (≥2yrs) pseudomembranous colitis post antibiotic treatment on all patients over 65 years old	Faeces	CCEY	35°C-37°C	Anaerobic	48-72hr	>48hr*	*Clostridium difficile*
* Cultures may be examined after overnight incubation, but should not be removed from the anaerobic cabinet because sporulation is inhibited on selective media, and young cultures may die on exposure to air. If using anaerobic jars, cultures must not be examined before 48 hours incubation							

4.6 Identification

4.6.1 Minimum level of identification in the laboratory

Clostridium difficile	'Species' level

Organisms may be further identified if this is clinically or epidemiologically indicated.

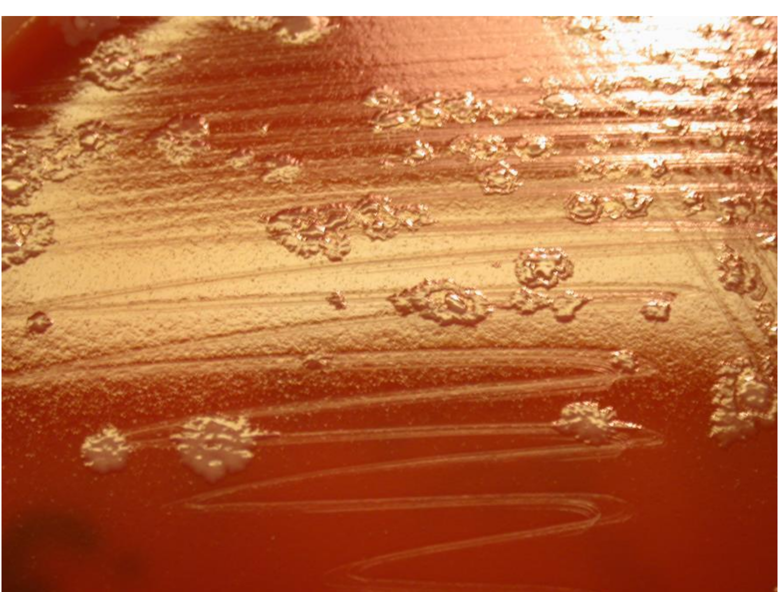
Colonies of *C. difficile* can be recognised by the following characteristics:

- If using egg-yolk based agar, a lack of opacity surrounding the colonies due to non-production of lecithinase (unlike *C. bifermentans*, *C. perfringens* or *C. sordellii*). Follow individual media manufacturer's guidelines on colonial morphology
- Green-yellow fluorescence under long-wave UV light (see below)
- Agglutination with *C. difficile* latex reagent for somatic antigen (see below)

For ease of identification, it is useful to sub-culture a putative *C. difficile* colony on FAA blood agar.

Examination of plates

Colonies of *C. difficile* may be smooth or rough, and may vary considerably in size. Typical colonies may be seen after sub-culture of suspect colonies from selective media on Fastidious Anaerobe Agar (See Plate 1):



Courtesy of Dr. Jon Brazier of the Anaerobic Reference Laboratory

Plate 1. Colonies of *C. difficile* on Fastidious Anaerobe Agar

Colonial Fluorescence

- Remove the test and control plates from the incubator and examine the colonies for fluorescence. Expose the colonies to long wave ultra-violet light (365nm) in a darkened room or light box held closely to the UV source and view by reflection

  Note: UV protective goggles must be worn.

- Colonies of *C. difficile* may vary in the intensity of fluorescence, but this will appear as a green-yellow or chartreuse colour. Fluorescence is poorly developed on some agar bases and is strongest on FAA. It is important to compare fluorescence of the test colonies with that of the control organisms to clarify positive and negative results. The colonial fluorescence of cultures >48hrs old on non-selective agars will diminish due to increased sporulation
- Mark any suspect (fluorescent) colonies on the underside of the plate with a felt tip pen. Sub-culture to a Fastidious Anaerobe Blood Agar (FAA) plate and incubate anaerobically for 48hrs

Note: Gram staining is rarely useful directly from selective agars; but from blood agar plates sub-terminal spores should be visible with most vegetative rods staining as Gram positive with some Gram variable forms in common with many other clostridial species. Routine Gram staining is not recommended in this SMI.

Latex agglutination test for somatic antigen

Use *C. difficile* somatic antigen latex agglutination and follow the instructions in the kit insert.

Limitations of the test

Cross-reactions with this reagent are known to occur with:

- *C. bifermentans*

- *C. sordellii*
- *C. glycolicum*

Controls

Set up controls alongside test cultures and on each new batch of medium (see 2.5).

Control organisms required:

- *C. bifermentans*
- *C. sordellii*
- *C. difficile*

Other clostridial species are commonly mistaken for *C. difficile.* These include *C. innocuum, C. glycolicum*, *C. bifermentans and C. sordellii*. However, these may be differentiated according to the criteria listed in Table 1.

Table 1. Differential tests for recognition of colonies of *C. difficile*

	C. difficile	***C. bifermentans***	***C. sordellii***	***C. glycolicum***	***C. innocuum***
UV (Fluorescence) at 365nm	+	-	-	-	+
Latex agglutination	+	+	+	+	-
Lecithinase on Brazier's CCEY medium	-	+	+	-	-

Organisms may be further identified if this is clinically or epidemiologically indicated.

Refer to individual SMIs for organism identification.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations

Isolates

Isolates from outside England (except Scotland) from situations that warrant typing investigations should be referred to the Anaerobe Reference Laboratory (ARL) for PCR ribotyping as detailed below. Isolates should only be referred for ribotyping following discussion and agreement with the designated laboratory.

With a charcoal transport swab, swab all the growth from a 48hr anaerobic culture of *C. difficile* on non-selective media such as FAA and replace swab in tube of transport medium.

Note: It is important to send growth from a 48 – 72hr culture to ensure sporulation.

Anaerobe Reference Laboratory (ARL)

NPHS Microbiology Cardiff
University Hospital of Wales
Cardiff
CF14 4XW

http://www.hpa.org.uk/cfi/arl/

Within Scotland, isolates from situations that warrant typing investigations should be referred to the Scottish *C. difficile* Reference Service for PCR ribotyping. Full contact details, referral criteria and isolate submission details are given on the website:

http://www.ssrl.scot.nhs.uk/cdiffservices.asp

Retention of isolates

When harvesting the growth from the FAA purity plate take a sweep of the growth and mix this in a fresh bijou of alcohol/saline (2:1) labelled appropriately. Store at -20°C. Alternatively, make a heavy suspension of the colonies in alcohol/saline (2:1) and store at -70°C.

Faeces

Within England there is now a *C. difficile* Ribotyping Network (CDRNE) consisting of six laboratories (in Leeds – reference centre, Birmingham, London, Manchester, Newcastle and Southampton) which is accessed in agreement with the relevant Regional Microbiologist. Access to this network should occur if a laboratory believes they have, or could have, a problem with an increased frequency or severity of cases of *C. difficile* infection; including increases in mortality, complications or recurrence rates. A standardised request form has been widely circulated in electronic format, which must be completed to access the service. Further details are available via the PHE website.

DH/PHE National *C. difficile* Surveillance Scheme

Referrals submitted as part of DH/PHE national *C. difficile* surveillance scheme (‘designated week’) should not use the Outbreak Investigation form but the separate one labelled “DH/PHE National *C. difficile* Surveillance Scheme”, PHE Regional Laboratories will request a pre-determined number of toxin positive stool samples within a given week from hospitals in their region rotation.

Northern Ireland *C. difficile* surveillance referral

Within Northern Ireland, the HSC Public Health Agency is responsible for routine *C. difficile* surveillance. Toxin-positive faeces for routine PCR ribotyping and outbreak investigations should be referred to the Belfast Health & Social Care Trust laboratory service. Contact details are given on the website: http://www.rvl-belfast.hscni.net/contact-us.

Antimicrobial susceptibility testing

The ARL is monitoring the antimicrobial susceptibilities of all isolates submitted under the DH/PHE surveillance scheme using the E test method for MIC determination to eight antibiotics. It is important that regular testing is done by all CDRNE laboratories to screen for any emerging resistance to the drugs of choice for treatment, namely metronidazole and vancomycin. Similar surveillance is performed in Scotland by the Scottish *C. difficile* Reference Service in conjunction with Health Protection Scotland (HPS).

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

N/A

5.2 Culture

Isolates of *C. difficile* submitted for typing investigations. Further report to follow.

5.3 Toxin Testing

C. difficile toxin detected **or**

C. difficile toxin not detected

5.4 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[65,66] or Equivalent in the Devolved Administrations[67-70]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/

Other arrangements exist in Scotland[67,68], Wales[69] and Northern Ireland[70].

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UK Standards for Microbiology Investigations

Investigation of swabs from skin and superficial soft tissue infections

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Bacteriology | B 11 | Issue no: 6.5 | Issue date: 19.12.18 | Page: 1 of 37

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Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 7

Scope of document 10

Key recommendations 10

Introduction 10

Technical information/limitations 20

1 Safety considerations 21

2 Specimen collection 21

3 Specimen transport and storage 22

4 Specimen processing/procedure 22

5 Reporting procedure 28

6 Notification to PHE, or equivalent in the devolved administrations 30

Appendix: Investigation of skin and superficial soft tissue infections 31

References 32

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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	14/19.12.18
Issue no. discarded.	6.4
Insert issue no.	6.5
Section(s) involved	**Amendment**
4.5.1 Culture media, conditions and organisms and appendix.	Minor amendment to table. Specifically, the culture information regarding anaerobes.

Amendment no/date.	13/01.05.18
Issue no. discarded.	6.3
Insert issue no.	6.4
Section(s) involved	**Amendment**
4.5.1 Culture media, conditions and organisms and appendix.	Minor amendment to table.

Amendment no/date.	12/01.03.18
Issue no. discarded.	6.2
Insert issue no.	6.3
Section(s) involved	**Amendment**
Introduction: Paronychia.	*Haemophilus influenzae* was removed.

Amendment no/date.	11/05.01.18
Issue no. discarded.	6.1
Insert issue no.	6.2

Section(s) involved	Amendment
4.5.1 Culture media, conditions and organisms	Minor amendment to table.

Amendment no/date.	10/08.08.16
Issue no. discarded.	6
Insert issue no.	6.1
Section(s) involved	**Amendment**
4.4.1	Section regarding Gram stain has been clarified.

Amendment no/date.	9/04.05.16
Issue no. discarded.	5.2
Insert issue no.	6
Section(s) involved	**Amendment**
Whole document.	Title updated to indicate sample type. References reviewed and updated throughout. Hyperlinks updated to gov.uk.
Scope.	Inclusion of swabs of pus. Inclusion of links to relevant SMIs.
Page 2.	Updated logos added.
Key recommendations.	Key recommendations included.
Introduction.	Original text reorganised and streamlined. Additional text included from B14 – Investigation of pus and exudates and B17 – Investigation of tissues and biopsies from deep-seated sites and organs following reorganisation of these documents.
Technical information/limitations.	Section of rapid methods included.
Specimen processing/procedure.	4.5.1 Culture media and organisms Specimen type added to table. All conditions – addition of Staph/Strep selective agar as an alternative to blood agar. Addition of

	CLED/MacConkey agar. Addition of swab of pus to supplementary media section. Removal of reference to swabs from dirty sites. Sabouraud agar incubation amended to 28-30ºC for 14d. 4.6.1 Minimum level of identification Aeromonas, dermatophytes and mould added to the table. Additional information included in right hand column regarding exceptions and information for specific situations. Information regarding *C. diphtheria* included. 4.7 Antimicrobial susceptibility testing Updated to include link to EUCAST and reference to CSLI. Antimicrobial susceptibility testing table included which recommends which antimicrobials should be tested and reported.
Reporting procedure.	Reporting procedure text updated in line with template.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratorieshttp://www.hpa-standardmethods.org.uk/. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

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laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Skin swab, swab from superficial, non-surgical and surgical wounds, and swab of pus

This SMI describes the processing of skin, superficial, non-surgical and surgical wound swabs, from sites accessible without intervention, for the microbiological investigation of skin and superficial soft tissue infections (SSTIs).

For pragmatic reasons the processing of swabs of pus has been included in this SMI. For further information regarding pus and exudate samples refer to B 14 – Investigation of pus and exudates.

It should be noted that many conditions are best diagnosed by submission of a skin biopsy for culture and histopathological examination (refer to B 17 - Investigation of tissues and biopsies from deep-seated sites and organs).

For information regarding dermatophyte infections see B 39 - Investigation of dermatological specimens for superficial mycoses.

Investigation of genital ulcers is dealt with in B 28 - Investigation of genital tract and associated specimens. Viruses such as herpes simplex and varicella-zoster, as well as parasites and non-microbial agents, may also cause skin lesions but are outside the scope of this SMI.

This SMI should be used in conjunction with other SMIs.

Key recommendations

Swabs are a diverse and heterogeneous group of specimens.

The specimen type and clinical details must therefore be taken into consideration when processing samples[1]. For example, swabs of pus should be investigated in a similar way to pus samples. In addition to the standard media recommended, supplementary media (ie fastidious anaerobic, cooked meat broth or equivalent) is also required for these samples. Refer to table 4.5.1.

A mechanism for urgent reporting should be in place to communicate key, clinically significant results in a timely manner.

Introduction

The skin is colonised by normally non-harmful flora. When the skin is broken as a result of trauma, burns, bites or surgical procedures, colonisation with a range of bacteria may occur[2]. Infections of the skin and subcutaneous tissues are caused by a wide range of organisms, however the majority are caused by *Staphylococcus aureus* and β haemolytic streptococci groups A, C and G[3,4].

Particular organisms are often typically associated with specific clinical conditions in skin and soft tissue infections, however overlaps in clinical presentation do occur[3,4]. Diagnosis is normally based on clinical presentation. Guidelines for diagnosis and management have been published which focus on a wide range of SSTIs from minor superficial to life threatening infections[5]. Microbiological cultures may be undertaken to

establish the causative organism enabling antibiotic sensitivity testing which is essential to ensure optimal treatment regimens.

Skin infections[2,4,6]

Cellulitis and erysipelas[7,8]

Cellulitis and erysipelas are diffuse spreading infections of the skin and subcutaneous tissue excluding cutaneous abscesses and necrotizing fasciitis[4]. Cellulitis involves the deeper layers of the skin and subcutaneous tissues, whereas erysipelas involves the upper dermis and superficial lymphatic system[4].

Cellulitis is commonly caused by[9,10]:

- β-haemolytic streptococci (including *Streptococcus pyogenes*)
- *S. aureus*

Wound infections may be caused by a broader range of organisms which, in addition to above, may include:

- *Bacteroides* species
- anaerobic cocci
- *Bacillus cereus*[11] (especially after trauma or orthopaedic surgery)
- enterobacteriaceae[12]

Superficial swabs in the absence of a skin break are often unrewarding; skin biopsies may produce better results but they are not frequently done. Recurrent cellulitis can occur following damage to local venous or lymphatic drainage systems[13,14].

Ecthyma gangrenosum

Ecthyma gangrenosum is a focal skin lesion characterised by haemorrhage, necrosis and surrounding erythema. It is usually caused by:

- *Pseudomonas aeruginosa*
- haematogenous dissemination of fungal infection (eg *Candida* species and mucoraceous fungi)[15,16]

Ecthyma gangrenosum may also rarely be caused by *Stenotrophomonas maltophilia*.

Similar lesions found in patients who are neutropenic may be due to infection with *Aspergillus* species or *Fusarium* species[17]. Diagnosis is usually based on clinical history and physical examination[9].

Impetigo

Impetigo is a superficial, intra-epidermal infection producing erythematous lesions that may be bullous or nonbullous[6]. Bullous impetigo is caused by *S. aureus*[4,18]. Nonbullous impetigo is most frequently caused by Lancefield Group A streptococci or *S. aureus*, and has occasionally been caused by streptococci of Lancefield Groups C and G[19].

Erysipelas

Erysipelas is a rare superficial infection of the skin[20]. It primarily involves the dermis and the most superficial parts of the subcutaneous tissues, with prominent

involvement of the superficial lymphatics. It presents as a painful, fiery red, oedematous area of skin, occasionally with small vesicles on the surface[4]. The margins have sharply demarcated, raised borders and the skin surface can appear orange peel like.

Erythrasma

Erythrasma is a common, chronic, superficial skin infection of the stratum corneum caused by *Corynebacterium minutissimum*. It presents with fine, scaly, reddish-brown plaques usually in the axillae and is often misdiagnosed as mycotic infection[21]. Diagnosis is most often made on clinical grounds rather than by culture.

Superficial mycoses

Superficial mycoses are cutaneous fungal infections that involve the hair or nails or the keratinized layer of the stratum corneum. A number of fungi can cause infection and are diagnosed through biopsy or aspirate. Normally skin scrapings are the specimens of choice (see B 39 - Investigation of dermatological specimens for superficial mycoses).

Causative organisms include[22]:

- dermatophytes
- *Candida* species
- Lipophilic yeasts

Paronychia

Paronychia is a superficial infection of the nail fold occurring as either an acute or chronic condition. Common isolates include[23]:

- *S. aureus*
- Lancefield Group A streptococci
- yeasts
- anaerobic bacteria

Folliculitis

Folliculitis is infection and inflammation of a hair follicle[24]. Dome-shaped papules or pustules form. These are each pierced by a hair and surrounded by a rim of erythema. The condition is usually caused by *S. aureus.*

Other possible causes include:

- *Pseudomonas aeruginosa*
  (can follow exposure in swimming pools or whirlpools)[25-28]
- *Candida* species
  (in patients receiving prolonged antibiotic or corticosteroid treatment)
- *Malassezia furfur*
  (in patients with diabetes or granulocytopenia or receiving corticosteroid treatment)[29,30]

Necrotising skin and soft tissue infections[4,10,31]

The terminology used for necrotising soft tissue infections is not consistent. Terms may relate to the kind of pathogen, the tissues involved, or the presence or absence of gas in the tissues[32,33].

It is clinically important to recognise these conditions as urgent surgical intervention, as well as antimicrobial therapy, is essential. Appropriate specimens are blood, fluid from bullae, and tissue biopsies. Growth from swabs taken from the surface of a lesion tends to be misleading, often yielding mixed cultures of colonising organisms. Mortality rates are high (30-60%)[33].

Gangrene

There are 4 main types of gangrene:

Meleney's progressive synergistic gangrene presents as a burrowing lesion or chronic gangrene of the skin usually following abdominal operations and results from mixed infections by organisms such as:

- *S. aureus*
- streptococci
- enterobacteriaceae
- pseudomonads
- anaerobic Gram negative bacilli[34]

Gas gangrene is a necrotising process associated with systemic signs of toxaemia and gas is present in the tissues. It often follows traumatic injuries such as penetrating wounds or crush injuries. Gas gangrene is caused by:

- *Clostridium perfringens*
- other *Clostridium* species

These organisms may however colonise a wound without causing disease. Alternatively, they may cause a spreading cellulitis, or extend into the muscle causing myonecrosis[10]. Classical gas gangrene is associated with clinical shock, leakage of serosanguinous fluid, tissue necrosis and presence of gas in the tissues.

Fournier's gangrene applies to the non-sporing anaerobes. These are particularly important causes of infection in the pelvic and scrotal areas, and are common causes of gangrene in ischaemic and diabetic limbs. They often occur in infections mixed with:

- enterobacteriaceae
- streptococci
- *Clostridium* species[35]

Spontaneous gangrene occurs either with no apparent relation to trauma or following mild, non-penetrating trauma. It is most commonly seen in patients with colonic carcinoma, leukaemia or neutropaenia. The main causative organisms are[36]:

- *C. perfringens*
- *Clostridium septicum*

Actinomycosis

Actinomycosis is a chronic suppurative infection characterised by abscess formation with the production of sulphur granules which mainly consist of micro-colonies of *Actinomyces* species[36]. Usual sites of infection are around the jaw, chest or abdomen. Material should be drained from these abscesses (B 14 - Investigation of deep-seated and organ, infections and abscesses) and biopsies taken (B 17 - Investigation of tissues and biopsies from deep-seated sites and organs).

Necrotising fasciitis[37,38]

Necrotising fasciitis is a serious, infrequently occurring infection primarily affecting the subcutaneous fat and superficial fascia of muscles and often the overlying soft tissues. The infection is most commonly caused by Group A streptococci. Swabs are not the sample of choice for the investigation of this infection (refer to B 17 - Investigation of tissues and biopsies from deep-seated sites and organs).

Myositis[39]

Myositis is not strictly within the scope of this document. It is an inflammation of the muscle which may be caused by bacterial, fungal or parasitic infection as well as non-infective conditions such as autoimmune disease or genetic disorders. Localised infection is usually due to bacteria or fungi, whereas viral and parasitic infections tend to be more diffuse. Necrotising myositis rapidly involves the entire muscle bed and may spread to adjacent tissues. Both polymicrobial and unimicrobial forms may be seen.

Pyomyositis is a purulent infection of skeletal muscle and occurs more commonly in tropical countries. It usually presents as a single abscess but multiple abscesses do occur. Most patients have no underlying predisposing condition, previous trauma accounting for only 25% of cases. The majority of cases are due to *S. aureus*. More rarely, fungi and viruses may cause infection in patients who are immunocompromised.

Mycetoma[40-43]

Mycetoma occurs in people living in tropical and sub-tropical climates, usually following a puncture wound. The condition results from a chronic destructive process involving the skin, subcutaneous tissue, muscle and bone. Granulation of tissue develops with chronic inflammation and fibrosis and is characterised by a draining sinus and the presence of granules. A mycetoma can form anywhere in the body, but is more common in the lower extremities. Formation in the foot is known as Madura foot.

Mycetomata are divided into two categories based on the aetiological agents involved; actinomycetoma caused by aerobic actinomycetes and eumycetoma caused by moulds. There are at least twenty moulds that may cause this condition; the species involved are often associated with distinct geographical areas.

Ninety five percent of the cases are caused by:

Eumycetoma:

- *Acremonium* species
- *Leptosphaeria senegalensis*
- *Madurella grisea*

- *M. mycetomatis*
- *Scedosporium* (*Pseudallescheria*) *apiospermum*
- *Pyrenochaeta romeroi*
- *Curvularia* species
- *Exophiala jeanselmei*
- *Phialophora verrucosa*

Actinomycetoma:

- *Actinomadura* species
- *Nocardia* species
- *Streptomyces* species
- *Madurella* species

Organisms are found in tissue sinuses as aggregates of filaments. These are called granules but differ from the sulphur granules of actinomycosis in that they do not have the characteristic clubbed peripheral fringe. Granules obtained directly from tissue will ensure the best cultural recovery of the causative organism because granules found in sinus discharge contain only dead organisms. Surgical biopsy to obtain material for culture is important for diagnosis, especially if sinus discharge is culture-negative for aerobic actinomycetes or is contaminated by other bacteria: the processing of tissue specimens in possible cases of mycetoma is described in B 17 - Investigation of tissues and biopsies from deep-seated sites and organs.

Carbuncles, foruncles, cutaneous, soft tissue and other abscesses[4]

Carbuncles are deep and extensive subcutaneous abscesses involving several hair follicles and sebaceous glands.

Foruncles are abscesses which begin in hair follicles as firm, tender, red nodules that become painful and fluctuant. Both carbuncles and foruncles are usually caused by *S. aureus*.

Cutaneous abscesses are usually painful, tender, fluctuant erythematous nodules often with a pustule on top. In some cases they are associated with extensive cellulitis, lymphangitis, lymphadenitis and fever. They are caused by a variety of organisms. The location of an abscess often determines the flora likely to be isolated. Thus *S. aureus* is most often isolated from cutaneous abscesses of the axillae, the extremities and the trunk, whereas cutaneous abscesses involving the vulva and buttocks may yield faecal or urogenital mucosal flora.

Burkholderia pseudomallei causes melioidosis, but is rare in the UK. The disease may present in a variety of forms with skin lesions and/or cellulitis. Diagnosis is made by blood culture, serology or culture of pus (refer to B 37 – Investigation of blood culture (for organisms other than *Mycobacterium* species)).

Abscesses in intravenous drug users

Cutaneous abscesses frequently occur as a complication of injecting drug use. They commonly result from the use of non-sterile solutions in which the drug is dissolved or from lubrication of the needle using saliva.

Bacterial isolates include[44]:

- oral streptococci
- *Streptococcus anginosus* group
- *Fusobacterium nucleatum*
- *Prevotella species*
- *Porphyromonas* species
- *S. aureus*
- *Clostridium* species
- *Bacillus anthracis*
(this is a rare but severe infection that can occur by injecting heroin contaminated with anthrax)[45]

Scalp abscess

Scalp abscesses are a recognised complication of electronic monitoring with fetal scalp electrodes during labour. A localised collection of pus surrounded by inflamed tissue forms where the electrodes are inserted. Anaerobes are most commonly isolated, probably as a result of contamination with vaginal organisms during delivery.

Polymicrobial infections also occur, involving[46]:

- anaerobes
- β-haemolytic streptococci
- *S. aureus*
- enterobacteriaceae
- enterococci
- coagulase negative staphylococci

Kerion is a pustular folliculitis of adjacent hair follicles, creating dense inflamed areas of the scalp, and is caused by dermatophytes (refer to B 39 – Investigation of dermatological specimens for superficial mycoses). Secondary bacterial infection may occur.

Ulcers

A skin ulcer is a lesion of the skin with loss of the skin integrity, which can extend from the epidermis down to deeper layers. There are various types of ulcers with different aetiology: pressure sores, diabetic foot ulcers, venous leg ulcers, arterial ulcers, autoimmune conditions such as pemphigus/pemphgoid. All ulcers are invariably colonised by a polymicrobial flora and microbiology samples should be taken only if a clinical diagnosis of infection has been made[47,48]. When swabs are taken from infected ulcers, they should be taken after cleansing and debridement: this aims at eliminating

part of the superficial colonising flora[48]. Sometimes chronic ulcer swabs are taken to identify the cause of underlying bone infections: in this scenario invasive bone biopsy specimens would be preferable, but ulcer swabs (after cleansing and debridement) are often taken in real practice but the results need careful interpretation[49].

Swabs from chronic non-healing ulcers or skin lesions with one of the following risk factors reported should be tested for *Corynebacterium* species:

- travel abroad to high risk area within the last 10 days
- contact with someone who has been to a high risk area within the last 10 days
- the patient works in a clinical microbiology laboratory, or similar occupation, where *Corynebacterium* species may be handled
- *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* can cause diphtheria and have been isolated from the skin of patients with chronic skin infections. For more information refer to ID 2 - Identification of *Corynebacterium* species)[21,50].

Burns[51,52]

Patients suffering from severe burns are at a higher risk of both local and systemic infection; sepsis is an important cause of mortality in this group of patients[51].

Organisms encountered include[51,53]:

- *S. aureus*
- β-haemolytic streptococci
- pseudomonads, especially *Pseudomonas aeruginosa*
- *Acinetobacter* species
- *Bacillus* species
- enterobacteriaceae
- filamentous fungi, eg: *Fusarium* species *and Aspergillus* species
- *Candida albicans,* non- *albicans Candida* species and other yeasts
- coagulase negative staphylococci

Gram negative organisms cause the most severe infections; fungal infections on the other hand can spread quickly, but are more easily treated, although a definitive diagnosis is difficult to obtain[51].

Bite wounds and contact with animals[4,54]

Bite wounds

Bite wounds can become contaminated by oral flora and normal human skin flora. Most bites are due to cats and dogs, but some are due to other pets (including reptiles, rodents and birds), domesticated animals (including horses, sheep etc) wild animals or other humans[4,54]. Organisms most commonly isolated include[4,55]:

- *Pasteurella multocida*
- *S. aureus*

- α-haemolytic streptococci
- streptococcus angiosus group

Other organisms associated with bite wounds which are rarely isolated include:

- anaerobes (including *Bacteriodes* species and Fusobacteria)
- *Capnocytophaga* species
- *Eikenella corrodens*
- *Haemophilus* species
- coagulase negative staphylococci
- *Streptobacillus moniliformis*
- *Staphylococcus intermedius*
- anaerobes (including Fusobacterium, Porphyromonas, Prerevotella etc)

Capnocytophaga canimorsus is associated with dog bites and causes septicaemia, particularly in patients with asplenia or underlying hepatic disease. This organism is usually isolated only from blood cultures.

Streptobacillus moniliformis is associated with rat bites and diagnosis is confirmed by culturing the organism from blood or joint fluid.

Other unusual organisms may be isolated including *Weeksella zoohelcum*, *Actinobacillus* species and *Neisseria canis*.

Insect bites are often associated with secondary Lancefield Group A streptococcus and *S. aureus* infection.

Contact with animals or animal products

Erysipeloid[56]

Erysipeloid is an uncommon nonsuppurative cellulitis due to *Erysipelothrix rhusiopathiae*. It is an occupational disease of fishermen, fish handlers, butchers and abattoir workers. It affects the hands and fingers causing lesions which present as painful purplish areas of inflammation with erythematous advancing edges.

Aeromonas and non-cholera *Vibrio* species

Aeromonas and non-cholera *Vibrio* species are predominantly isolated from traumatic water-related wounds or lacerations received whilst swimming in fresh or salt water, from other environmentally contaminated wounds, or from fishing or shellfish inflicted injuries[57-59]. Aeromonas infection may also follow the therapeutic use of leeches[60,61]. Water-related injuries can be polymicrobial involving environmental Gram negative organisms such as *Edwardsiella tarda* and pseudomonads[62].

Bacillus anthracis

Bacillus anthracis is the causative agent of anthrax which appears clinically in one of several forms; cutaneous (skin) anthrax or inhalation anthrax, as well as, more recently, injective anthrax[45]. Following the deliberate release of *B. anthracis* in the USA in 2001, there has been an increased awareness of the release of this and other organisms which may pose a biological threat. Cutaneous anthrax occurs through inoculation of spores to the skin or by contamination of abrasions. Skin lesions known

as malignant pustules develop, which are characteristic ulcers with a black centre[63]. They are rarely painful, but if untreated the infection can spread to cause septicaemia. If untreated, the disease can be fatal in 5% of cases, but with antibiotic treatment recovery is usual. Cutaneous infection with *B. anthracis* can occur in industrial workers who use materials of animal origin such as wool, leather, bristles and fur, or in the agricultural workplace for example farmers, husbandmen, butchers and vets. In rare cases *B. anthracis* has been transmitted via insect bites[64].

Other skin infections[4]

Skin infections may also be caused by the following:

- MRSA may colonise and/or infect wounds and soft tissue[65]. Newly emerging community (mecIV) MRSA with virulence factors such as Panton-Valentine Leukocidin (PVL) or Scalded Skin Toxin (SST) cause highly contagious infections such as follicultis in healthy children and young adults[66,67]. Infections are often spread through poor hygiene[68]. Panton-Valentine Leukocidin (PVL) is a toxin which is capable of destroying white blood cells[67]. Scalded skin syndrome (Lyell's syndrome in older children; Ritter's syndrome in infants) is caused by *S. aureus* phage types group II and 71[69]
- *Mycobacterium* species can cause cutaneous infections[70]. These may signify a disseminated systemic infection or may represent a local infection by non-tuberculous mycobacteria (see B 40 - Investigation of specimens for *Mycobacterium* species)
- rapid growing mycobacterial strains such as *M. chelonae* and *M. fortuitum* have also been isolated from superficial skin infections[71]. *M. chelonae* has been shown to be associated with tattoo related infections[72]
- *Sporothrix schenkii* causes sporotrichosis[73]. Cutaneous sporotrichosis is acquired by contamination with soil, sphagnum moss or other vegetable matter and develops at the site of inoculation to form a primary lesion with lymphatic spread (see B 39 - Investigation of dermatological specimens for superficial mycoses). It is more common in warmer climates
- cutaneous salmonellosis and listeriosis may also occur in veterinarians and farmers, typically on the arms, following assisted delivery of farm animals, usually cattle infected in utero[74,75]. Cutaneous listeriosis in a patient with AIDS has also been reported[76]
- *Yersinia enterocolitica* can cause cutaneous infections[77]

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedure

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[78,79]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Anaerobic plate incubation

The recommended incubation time for anaerobic plates is 48 hours. However some anaerobic bacteria such as certain species of *Actinomyces* require longer incubation (7 days) and will not be detected if plates are examined sooner.

Rapid methods

To reduce turnaround times, rapid identification and sensitivity tests may be performed in conjunction with routine methods where appropriate. A variety of rapid identification and sensitivity methods have been evaluated; these include molecular techniques and the Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[80]. It is important to ensure that fresh cultures of pure single isolates are tested to avoid reporting misleading results.

Laboratories should follow manufacturers' instructions and all rapid tests must be validated and be shown to be fit for purpose prior to use.

1 Safety considerations[78,79,81-95]

1.1 Specimen collection, transport and storage[78,79,81-84]

Use aseptic technique.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[78,79,81-95]

Containment Level 2.

If infection with a Hazard Group 3 organism, eg *Bacillus anthracis* (cutaneous anthrax is rare but needs to be recognised as a possibility in certain settings such as exposure to animal hides, injection of contaminated heroin in IVDUs and bioterrorist events such as the dissemination of spores in letters that took place in the USA in 2001), all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[87].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Skin swab, swab from superficial, non-surgical and surgical wounds, swabs of pus

2.2 Optimal time and method of collection[96]

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[96,97].

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[98-102].

Samples of pus/exudate, if present, are preferred to swabs (see B 14 – Investigation of deep-seated and organ, infections and abscesses). If only a minute amount of pus or exudate is available it is preferable to send a pus/exudate swab in transport medium to minimise the risk of desiccation during transport.

Sample a representative part of the lesion[97,103]. Swabbing dry crusted areas is unlikely to yield the causative pathogen[103].

If specimens are taken from ulcers, the debris on the ulcer should be removed and the ulcer should be cleaned with saline. A biopsy or, preferably, a needle aspiration of the edge of the wound should be taken[48].

A less invasive irrigation-aspiration method may be preferred. Place the tip of a small needleless syringe under the ulcer margin and irrigate gently with at least 1mL sterile

0.85% NaCl without preservative. After massaging the ulcer margin, repeat the irrigation with a further 1mL sterile saline. Massage the ulcer margin again, aspirate approximately 0.25mL of the fluid and place in a CE marked leak proof container[104].

Fungal specimens for dermatophytes: See B 39 - Investigation of dermatological specimens for superficial mycoses.

2.3 Adequate quantity and appropriate number of specimens[96]

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

3 Specimen transport and storage[78,79]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[96].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[96].

4 Specimen processing/procedure[78,79]

4.1 Test selection

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

N/A

4.3.2 Specimen processing

See Q 5 - Inoculation of culture media for bacteriology.

4.4 Microscopy

4.4.1 Standard

Gram stain is not normally required. However, Gram films should be considered from pus swabs if they originate from severe deep seated infections.

4.4.2 Supplementary

See B 40 - Investigation of specimens for *Mycobacterium* species, and TP 39 - Staining procedures.

4.5 Culture and investigation

Inoculate each agar plate directly by rolling the swab on a part of the plate or by using a sterile pipette (Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
All conditions	Swabs	Blood agar And/or Staph/Strep selective agar	35-37 35-37	5 -10% CO_2 Air	40-48hr 40-48hr	Daily Daily	Top pathogens: Lancefield Groups A, C and G streptococci *S. aureus* In specific circumstances e.g. bites or exposure to animals and animal products or fresh/salt water (use blood agar): *Pasteurella* species *Vibrio* species *Aeromonas* species *Bacillus cereus*/anthracis *Strep. pneumoniae* *Eikenella corrodens* Capnocytophaga Erysipelothrix
		CLED/MacConkey agar	35-37	Air	18-24hr	>18hr	Clinical circumstances determines significance of the following isolates Enterobacteriaceae Pseudomonads
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Wound swabs eg traumatic wounds	Swabs	Selective anaerobe agar with metronidazole 5µg disc	35-37	Anaerobic	5d	≥40hr and at 5d $^{+}$	Anaerobes
Swab of pus	Swabs	Fastidious anaerobic, cooked meat broth or equivalent Subculture to BA if evidence of growth (≥40hr), or at day 5	35-37 35-37	Air As above	5d 40-48hr	N/A ≥40hr	Any organism
Cellulitis in children Human bites	Swabs	Chocolate agar †	35-37	5-10% CO_2	40-48hr	daily	Fastidious organisms *Haemophilus* species

Burns Patients who are Immunocompromised Diabetic patients Intertrigo Paronychia*	Swabs	Sabouraud agar	28-30	Air	14 d	daily	Yeast Mould
Suspected cutaneous diphtheria (Consider for foreign travel with <10 d and non-healing ulcers)	Swabs	Hoyle's tellurite agar	35-37	Air	40-48hr	daily	*C. diphtheriae* *C. ulcerans*
Other organisms for consideration: Dermatophytes (B 39 - Investigation of dermatological specimens for superficial mycoses) and *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species)							
[+] Some anaerobic bacteria such as certain species of Actinomyces require longer incubation (7 days) and will not be detected if plates are examined sooner. † Either bacitracin 10 unit disc or bacitracin - containing agar may be used. * Will need a layer of oil to culture for mould							

4.5.2 Supplementary investigations

Toxigenicity testing of *C. diphtheriae.*

See B 40 - Investigation of specimens for *Mycobacterium* species.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Aeromonas	species level
Anaerobes	anaerobes level except in necrotising infections
Bacillus species	species level when appropriate to diagnose or exclude *B. anthracis* or *B. cereus* infections
β-haemolytic streptococci	Lancefield Group level
Coagulase negative staphylococci	coagulase negative level
C. diphtheriae	species level and urgent (same-day) toxigenicity test (when appropriate clinical details)
C. minutissimum	species level in erythrasma
C. ulcerans	species level (when appropriate clinical details)
Dermatophytes	B 39 - Investigation of dermatological specimens for superficial mycoses
E. corrodens	species level

Enterobacteriaceae	coliforms level except in necrotising infections
E. rhusiopathiae	species level
Haemophilus	species level
Mould	genus level
Pasteurella	species level
Pseudomonads	Usually only at pseudomonads level except in echtyma gangrenosum, recreational water folliculitis, necrotising infections, burns
S. aureus	species level (consider Panton-Valentine leukocidin (PVL) and toxin testing if appropriate clinical details)
S. pneumoniae	species level
Yeasts	yeasts level
Vibrio	species level

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: All work on suspected isolates of *C. diphtheriae* which is likely to generate aerosols must be performed in a safety cabinet[85].

A medical microbiologist must be informed of all suspected isolates of *C. diphtheriae* as soon as possible (same-day toxigenicity testing is available from the reference laboratory).

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC), EUCAST and/or CSLI guidelines or manufacturer's validation for proprietary methods.

This SMI recommends selective and restrictive reporting of susceptibilities to antimicrobials. Any deviation must be subject to consultation that should include local antimicrobial stewardship groups.

4.7.1 Antimicrobial susceptibility testing and reporting table

It is recommended that the antimicrobials in bold in the table below are reported. Those antimicrobials not in bold may be reported based on local decisions.

For more information on Detection of bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases) refer to B 60.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
S. aureus	**Cefoxitin[1] (or Oxacillin)** **Erythromycin/ Clarithromycin** **Tetracycline[2]**	Clindamycin Co-trimoxazole Daptomycin Fusidic acid Gentamicin Linezolid Mupirocin Penicillin Rifampicin Teicoplanin Vancomycin	1. Report as Flucloxacillin. 2. Supress report in children.
Pyogenic Streptococci	**Erythromycin/ Clarithromycin** **Penicillin** **Tetracycline[2]**	Clindamycin Co-trimoxazole Linezolid Vancomycin	2. Supress report in children.
Enterobacteriaceae from clean surgical sites	Ampicillin (or Amoxicillin) Cefpodoxime[4] Co-amoxiclav[5] Gentamicin	Amikacin Aztreonam Cefotaxime (or Ceftriaxone) Ceftazidime Cefuroxime Ciprofloxacin Co-trimoxazole Ertapenem Meropenem (or Imipenem) Piperacillin/Tazobactam Temocillin	3. Antibiotics should only be reported in the presence of clinical evidence of infection. 4. Cefpodoxime resistant organisms should be tested for the presence of ESBLs and screened for reduced susceptibility to carbapenems. 5. Co-amoxiclav resistant organisms should be tested at local level for sensitivity to carbapenems.

Enterobacteriaceae from sites prone to colonisation (eg ulcers)		Amikacin Ampicillin (or Amoxicillin) Aztreonam Cefpodoxime[4, 6] Cefuroxime Ciprofloxacin Ceftazidime Cefotaxime (or Ceftriaxone) Co-amoxiclav[5, 6] Cotrimoxazole Ertapenem Gentamicin Meropenem (or Imipenem) Piperacillin/Tazobactam Temocillin	4. Cefpodoxime resistant organisms should be tested for the presence of ESBLs and screened for reduced susceptibility to carbapenems. 5. Co-amoxiclav resistant organisms should be tested at local level for sensitivity to carbapenems. 6. If susceptibility testing is being undertaken, include this agent.
Pseudomonads		Amikacin Ceftazidime Ciprofloxacin Gentamicin Meropenem (or Imipenem) Piperacillin/Tazobactam	

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Standard

Gram stain (not usually required)

Report on WBCs and organisms detected.

Supplementary

For the reporting of microscopy for *Mycobacterium* species refer to B 40 – Investigation of specimens for *Mycobacterium* species.

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

Following results should be reported:

- clinically significant organisms isolated
- other growth
- absence of growth

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

Refer to table 4.7.1. The table includes guidance on the minimum range of agents that should be tested on the bacterial isolates listed. The table also includes additional agents that can be considered for inclusion in test panels in specific clinical scenarios.

Any deviation from the guidance should be subject to local consultation and risk assessment.

Generally, all resistant results should be reported as this is good practice and informs the user.

6 Notification to PHE[105,106], or equivalent in the devolved administrations[107-110]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

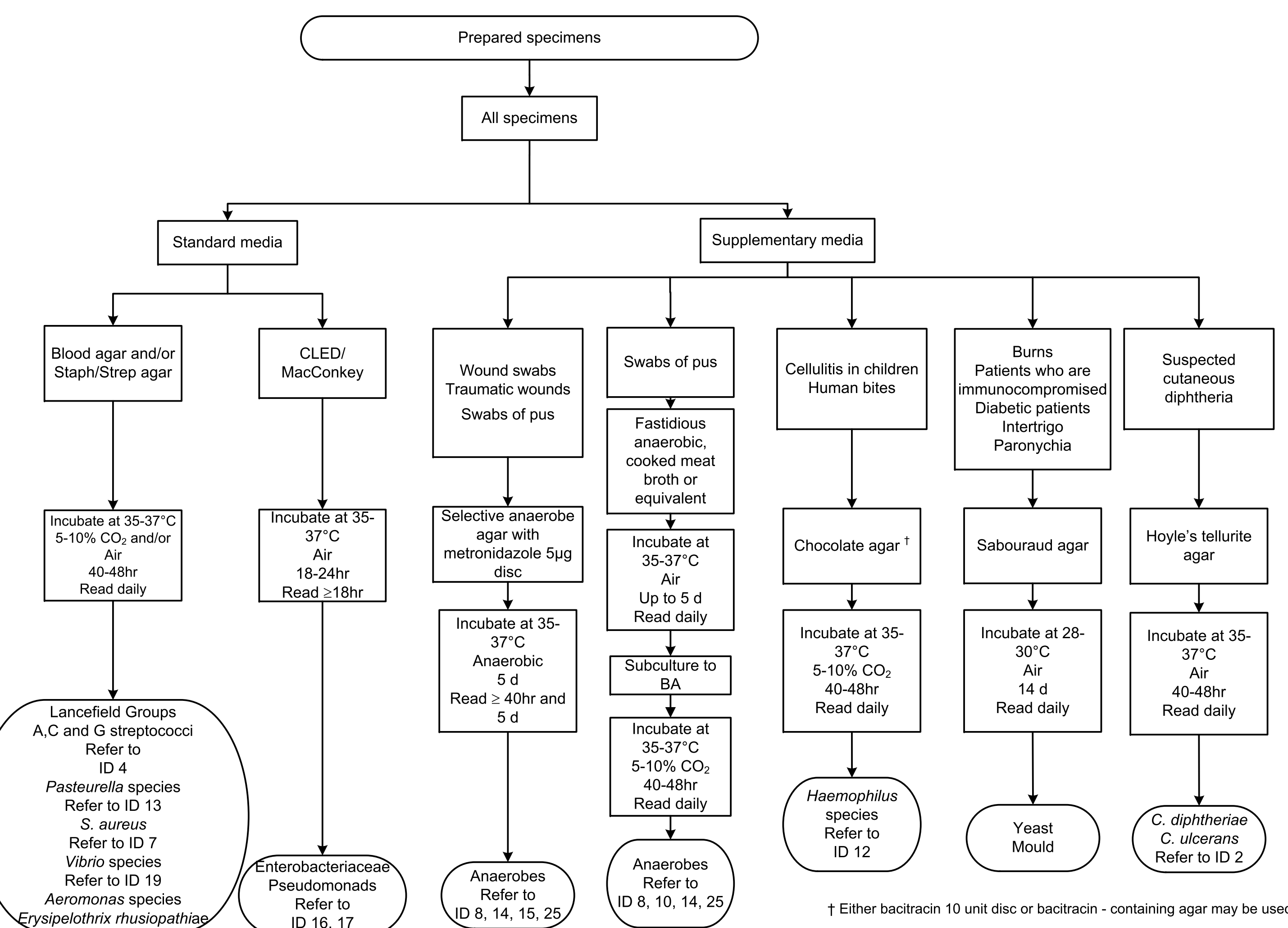
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[107,108], Wales[109] and Northern Ireland[110].

Appendix: Investigation of skin and superficial soft tissue infections



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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 11 Investigation of swabs from skin and superficial soft tissue infections

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation: 06/01/2015 – 26/01/2015

Version of document consulted on: B 11dn+

Proposal for changes

Comment number	1		
Date received	06/01/2015	**Lab name**	Microbiology Queen Elizabeth Hospital
Section	Introduction Page 9		
Comment			
Erythrasma section, 3rd line 1st paragraph on page 'my mycotic' typo error.			
Financial barriers			
N/A			
Health benefits			
No.			
Recommended action	**ACCEPT** Text updated.		

Comment number	2		
Date received	26/01/2015	**Professional body**	IBMS
Section	a. Introduction - Cellulitis and Erysipelas b. Whole document c. Introduction - Erythrasma d. Section 4.6.1 e. Technical Information/Limitations – Specimen Containers f. Section 4.7		
Comment			
a. *Mycoplasma phocacerebrale* should be considered as a potential cause of cellulitis and/or adding to the animal bite section. This organism has been documented as the cause of cellulitis from animal bites in handlers of marine animals. There is a potential to confuse such infections with *Erysipelothrix* resulting in potential treatment failures (see evidence paper). b. Bacterial names need to be italicised throughout, not complete throughout the document. c. Under Erythrasma; 3 line Erroneous text 'my' in sentence "plaques usually in the axillae and is often misdiagnosed as my mycotic infection."			

<table>
<tr><td colspan="2">
d. Line in table. If a yeast is significant in a site surely it should be identified, especially if treatment is to be given as antifungal break points are species specific.
e. Under the specimen containers section it mentions that CE marked leak proof containers should be used, but there is no reference to M40 complaint swabs (B11 and B14 only) despite stating that samples on swabs were acceptable for investigation. The CLSI M40-A2 Quality Control of Microbiological Transport Systems was revised in June 2014 and is the expected standard for transport swabs.
f. Under the antimicrobial susceptibility testing each document make reference to BSAC or EUCAST which is fine for bacterial pathogens. However, for Candida and Moulds (which are mentioned in the text) only CLSI breakpoints apply.
</td></tr>
<tr><td colspan="2">Evidence</td></tr>
<tr><td colspan="2">
a. http://www.ncbi.nlm.nih.gov/pubmed/21119845
www.bdmlr.org.uk/uploads/documents/resources/bdmlr-seal-bites.doc
</td></tr>
<tr><td>Recommended action</td><td>
a. NONE
A literature search was carried out on Pubmed and18 references were identified regarding Seal finger. Of these two were case reports in English regarding Mycoplasma phocacerebrale. It was therefore agreed, that as this is rarely reported, it would not be included in the document.
b. ACCEPT
Text updated.
c. ACCEPT
Text updated.
d. ACCEPT
It was agreed that ‘yeast’ level was satisfactory as a minimum level of identification for yeast in this document. Further identification can be performed where clinically indicated. The fungal information in the introduction will be updated for consistency.
e. NONE
CLSI M40 – A2 Quality control of microbiological transport systems is a quality standard not enforceable within the UK. The standard is for manufacturers and it is therefore outside of the scope of this document. The standard will therefore not be included in the SMIs.
f. NONE
Antimicrobial susceptibility break points for different species of yeast are available from EUCAST, however they are not required in this document as yeast are identified to yeast level only. Therefore a reference to CSLI will not be included in this SMI.
</td></tr>
</table>

2nd Consultation: 07/09/2015 – 05/10/2015

Version of document consulted on: B 11dw+ 07/09/2015 – 24/09/2015

B 11dy+ 25/09/2015 – 05/10/2015

Proposal for changes

Comment number	1		
Date received	08/09/2015	**Lab name**	Jersey General Hospital
Section	AST 4.7.1		
Comment			
It states that tetracycline vs *S. aureus* may be suppressed in children. Should tetracycline vs β haemolytic streptococci also follow this rule?			
Financial barriers			
Possible barriers from consultants who had traditionally more antimicrobial susceptibility testing options provided & to ensure that antibiotics reported ties with local policy.			
Recommended action	**ACCEPT** Text in table updated.		

Comment number	2		
Date received	11/09/2015	**Lab name**	Salford Royal NHS Foundation Trust
Section	4.7.1 Antimicrobial Susceptibility Testing and Reporting Table		
Comment			
a. *S. aureus* row - Penicillin: Only 10% susceptible is this really good use of a disc? b. *S. aureus* row - Clindamycin: Add co-trimoxazole c. Pyogenic Streptococci row - Clindamycin: Add linezolid? d. *Enterobacteriaceae* from surgical sites row - Amikacin: Add co-trimoxazole as oral option e. *Enterobacteriaceae* from surgical sites row - Ciprofloxacin: Move to first line (as beta-lactam allergy option) f. *Enterobacteriaceae* from surgical sites row - Cefotaxime: Should this be cefoxitin? g. *Enterobacteriaceae* from sites prone to colonisation (eg ulcers) row - Ampicillin: Add co-trimoxazole as oral option h. *Enterobacteriaceae* from sites prone to colonisation (eg ulcers) row - Ciprofloxacin: Move to first line (as option for penicillin allergy) i. *Enterobacteriaceae* from sites prone to colonisation (eg ulcers) row - Cefotaxime:			

	Should this be cefoxitin? j. Pseudomonads row - Cefuroxime: Unlikely to be active vs Pseudomonads
Recommended action	a. **ACCEPT** Penicillin moved to the primary testing panel. b. **ACCEPT** Co-trimoxazole has been added to the primary testing panel. c. **ACCEPT** Linezolid has been added to the primary testing panel. d. **ACCEPT** Co-trimoxazole was included, in the second version of the document to go for consultation, in the supplementary testing panel. e. **NONE** It was agreed that ciprofloxacin should remain in the supplementary testing panel. f. **NONE** Cefoxitin is used infrequently in the UK. Note 6 regarding AmpC removed. g. **ACCEPT** Co-trimoxazole added to the supplementary testing panel. h. **NONE** It was agreed that ciprofloxacin should remain in the supplementary testing panel. i. **NONE** Cefoxitin is used infrequently in the UK. Note 6 regarding AmpC removed. j. **ACCEPT** This was included in error and was removed from the second version of the document that went for consultation.

Comment number	3		
Date received	14/09/2015	**Lab name**	Professional
Section	Page 12 & 15		
Comment			
Typos: a. Page 12: Furuncles instead of foruncles. b. Page 15: Prevotella instead of prerevoltella.			

Recommended action	a. **ACCEPT** Text updated. b. **ACCEPT** Text updated.

Comment number	4		
Date received	24/09/2015	**Lab name**	NHS Highland-Oban Laboratory
Section	4.7.1		
Comment			
Enterobacteriaceae from surgical site? Cefotaxime as indicator of AmpC production. Is cefoxitin a better antibiotic for the non-specific differentiation of AmpC activity from ESBL activity? Notes 6.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**PARTIAL ACCEPT** It was agreed that cefoxitin is a better antibiotic for use as an indicator of AmpC production. However, cefoxitin is used infrequently in the UK and therefore Note 6 regarding AmpC has been removed.		

Targeted questions:

Do you agree with the concept of including antimicrobial susceptibility testing and reporting tables in SMIs?		
Date received	**Lab name**	**Comment**
08/09/2015	Jersey General Hospital	Yes - if the data is generated by a reputable source ie EUCAST and does not contradict what the sources website/other literature state then that's helpful to me.
14/09/2015	Professional	Yes.
24/09/2015	NHS Highland-Oban Laboratory	Yes.

Do you agree with the content of the antimicrobial susceptibility testing and reporting table in this SMI?		
Date received	**Lab name**	**Comment**
08/09/2015	Jersey General Hospital	Yes.
14/09/2015	Professional	Yes.
24/09/2015	NHS Highland-Oban Laboratory	Add temocilin for potential identification of CPE producers?

Comments received outside of consultation

Comment number	1		
Date received	02/02/2015	**Professional body**	ACOM
Section	Various		
Comment			

Under consultation document

a. Introduction

Fungal infections are certainly also very common! Suggest you add at least dermatophytes here.

b. Mycetoma page 11

Change mould to moulds.

c. Ulcers page 13

Please add viral infections, dermatological conditions (lichen) and autoimmune conditions (pemphigus/pemphgoid).

d. Bite wounds page 15

Add 'and Strep anginosus group' to 'a-haemolytic streptococci'.

e. Section 4.5.1

Add fungi to the table and the flowchart.

f. Section 4.5.1

Fastidious organisms: oral streps and anaerobes.

g. Section 4.6.1

Moulds need to be added.

Under review document

h. Introduction

<table>
<tr><td colspan="2">
Need to add the main fungal pathogens (such as dermatophytes, Candida).
i. Superficial mycoses page 12
Need to add mould infections of the nails.
j. Other skin infections page 13
Should systemic bacterial infections be mentioned (eg meningococcal sepsis) as systemic mycoses are?
k. Section 4.5.3
Haemophilus species: Oral streps and anaerobes missing.
l. Section 4.5.3
Fungi: Yeasts, moulds and dermatophytes? Other targets are given genus/species level. Would be helpful to expand “fungi”.
</td></tr>
<tr><td>Recommended action</td><td>
a. PARTIAL ACCEPT
Link to the dermatophyte SMI added to the scope.
b. ACCEPT
Text updated.
c. PARTIAL ACCEPT
Viral infections, dermatological conditions (lichen) are outside of the scope of the document. Text updated to include pemphigus/pemphgoid.
d. ACCEPT
Streptococcus angiosus group added to the list or organisms.
e. NONE
It was agreed that fungi would not be added to the flowchart, yeasts and moulds are included in the table. The list of organisms is not comprehensive, only the most common organisms isolated are included.
f. NONE
It was felt the fastidious organisms (oral streptococci and anaerobes) were already sufficiently covered in B4 - Investigation of mouth swabs and did not need to be added to this document.
g. ACCEPT
Table updated to include moulds.
h. ACCEPT
Fungal infections included throughout introduction.
i. PARTIAL ACCEPT
Text updated and link to B 39 - Investigation of dermatological specimens for superficial mycosis included.
j. NONE
</td></tr>
</table>

	It was felt that oral streptococci and anaerobes were already sufficiently covered in B4 - Investigation of mouth swabs and did not need to be added to this document. k. **PARTIAL ACCEPT** It was agreed that mould should be identified to 'genus' level and yeasts to 'yeast' level. l. **PARTIAL ACCEPT** Systemic bacterial infections in relation to *Mycobacterium* species infection and burns patients included.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 5			
Date received	15/01/2015	**Lab name**	Nottingham University Hospitals
Date received	21/01/2015	**Lab name**	Northern Health and Social Care Trust
Date received	23/01/2015	**Lab name**	Truro
Date received	14/09/2015	**Lab name**	Microbiology, Northern Health and Social Care Trust
Date received	02/10/2015	**Lab name**	Microbiology at Hairmyres Hospital

UK Standards for Microbiology Investigations

Investigation of pus and exudates

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 14 | Issue no: 6.2 | Issue date: 22.11.16 | Page: 1 of 34

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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Logos correct at time of publishing.

Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 6

Scope of document 9

Key recommendations 9

Introduction 9

Technical information/limitations 17

1 Safety considerations 19

2 Specimen collection 19

3 Specimen transport and storage 20

4 Specimen processing/procedure 20

5 Reporting procedure 25

6 Notification to PHE, or equivalent in the devolved administrations 27

Appendix: Investigation of pus and exudates 28

References 29

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	10/22.08.16
Issue no. discarded.	6.1
Insert issue no.	6.2
Section(s) involved	**Amendment**
4.5.1.	Incubation temperature and atmosphere on clinical conditions "*Nocardiosis*" has been updated.
Appendix.	Updated to reflect section 4.5.1 culture media, conditions and organisms table.

Amendment no/date.	9/08.08.16
Issue no. discarded.	6
Insert issue no.	6.1
Section(s) involved	**Amendment**
4.3.2.	Section regarding swabs has been clarified.

Amendment no/date.	8/20.06.16
Issue no. discarded.	5.2
Insert issue no.	6
Section(s) involved	**Amendment**
Title.	Changed to 'investigation of pus and exudates'.
Whole document.	References reviewed and updated.
Type of specimen.	Pus or exudate.
Key recommendations.	Key recommendation section included.
Introduction.	Document streamlined and re-structured.

	Information regarding skin related abscesses and post-operative wound infections removed and transferred to B 11 - Investigation of swabs from skin and superficial soft tissue infections. Inclusion of throat and neck abscesses.
Technical information /limitations.	Inclusion of information on selective media and rapid methods.
Safety considerations.	Recommendations included regarding the use of Class I or Class II microbiological safety cabinets where Gram negative coccobacilli are identified.
Specimen collection.	Samples of pus are preferred to swabs.
Culture and investigation.	Section 4.5.1 - Table updated to include specimen type. Neomycin fastidious anaerobic agar replaced by selective anaerobic agar for all specimens. Cooked meat broth or equivalent added as alternative to fastidious anaerobic broth. GN Medium (NAV) replaced with selective Gram negative anaerobe medium. Minor changes to incubation and culture reads throughout for consistency. Section 4.6 - Minimum level of identity updated for the following organisms: anaerobes, β-haemolytic streptococci, enterobacteriaceae and yeast. Consider sending staphylococci isolates from post mortem samples for toxin testing. Section 4.7 - Antimicrobial susceptibility testing section updated. Recommendations for selective reporting are not included. Section 4.9 - Consider sending *S. aureus* isolates for toxin testing where appropriate clinical details are provided.
Reporting procedure.	Updated in line with bacteriology template. Report antimicrobial susceptibilities as clinically indicated.
Appendix.	Updated to reflect section 4.5.1 culture media, conditions and organisms table.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user’s risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

Public Health England. (2016). Investigation of pus and exudates. UK Standards for Microbiology Investigations. B 14 Issue 6.2. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Pus, exudate

This SMI describes the processing and microbiological investigation of pus and exudate specimens from abscesses and infections which are deep seated or associated with specific organs (including the skin).

Investigations of these specimens for parasitic infections are in B 31 - Investigations of specimens other than blood for parasites, whereas investigations for mycobacterial cultures are in B 40 – Investigations of specimens for *Mycobacterium* species.

For information regarding genital tract abscesses and infections refer to B 28 – Investigation of genital tract and associated specimens.

For information on skin and soft tissue infections and regarding testing of swabs of pus refer to B 11 - Investigation of swabs from skin and superficial soft tissue infections.

This SMI should be used in conjunction with other SMIs.

Key recommendations

Samples should be transported to the laboratory and processed rapidly.

Gram stain should be undertaken on all samples.

Gram stain results should be taken into consideration when choosing which investigations to carry out.

Significant microscopy, culture and sensitivity results should be issued as soon as they become available.

A mechanism for urgent reporting should be in place to communicate key, significant results.

Introduction

Abscesses are accumulations of pus in tissue and any organism isolated from them may be of significance. They occur in many parts of the body as superficial infections or as deep-seated infections associated with any internal organ. Many abscesses are caused by *Staphylococcus aureus* alone, but others are mixed infections. Anaerobes are predominant isolates in intra-abdominal abscesses and abscesses in the oral and anal areas. Members of the "*Streptococcus anginosus*" group and Enterobacteriaceae are also frequently present in lesions at these sites.

Bartholin gland abscesses and tubo-ovarian abscesses are considered in B 28 – Investigation of genital tract and associated specimens. Processing of specimens for *Mycobacterium* species, for example from subcutaneous cold abscesses, is described in B 40 – Investigation of specimens for *Mycobacterium* species.

Brain abscess[1-4]

Brain abscesses are serious and life-threatening.

Sources of abscess formation include[5]:

- direct contiguous spread from chronic otitic or paranasal sinus infection
- metastatic haematogenous spread either from general sepsis or secondary to chronic suppurative lung disease
- penetrating wounds
- surgery
- cryptogenic (ie source unknown)

Brain abscesses of dental origin are rare[6]. The mortality rate of these abscesses is high even when appropriately treated[7].

Treatment of brain abscesses involves the drainage of pus and appropriate antimicrobial therapy[5]. Brain stem abscesses have a poor prognosis due to their critical anatomical location[8].

Bacteria isolated from brain abscesses are usually mixtures of aerobes and obligate anaerobes, and the prevalent organism may vary depending upon geographical location, age and underlying medical conditions. The most commonly isolated organisms include[9-11]:

- anaerobic streptococci
- anaerobic Gram negative bacilli
- "*Streptococcus anginosus*" group
- Enterobacteriaceae
- *Streptococcus pneumoniae*
- β-haemolytic streptococci
- *S. aureus*

Organisms commonly isolated vary according to the part of the brain involved. Many other less common organisms, for example *Haemophilus* species, may be isolated[9,10,12]. *Nocardia* species often exhibit metastatic spread to the brain from the lung. Any organism isolated from a brain abscess must be regarded as clinically significant.

Organisms causing brain abscesses following trauma may often be environmental in origin, such as *Clostridium* species or skin derived, such as staphylococci and *Propionibacterium* species[13].

Brain abscesses due to fungi are rare. Aspergillus brain abscess can occur in patients who are neutropenic. Zygomycosis (mucormycosis) is an uncommon opportunistic infection caused by *mucoraceous moulds, for example Lichtheimia (formerly Mycocladus, Absidia)* and related fungi. *Scedosporium apiospermum* (*Pseudallescheria boydii*) enters the body and spreads haematogenously[14].

The use of culture based methods for organism identification is time consuming; molecular tests are becoming popular resulting in improved management of brain abscesses[3].

Breast abscess

Breast abscesses occur in both lactating and non-lactating women. In the former, infections are commonly caused by *S. aureus*, but may be polymicrobial, involving anaerobes and streptococci[7,15]. Signs include discharge from the nipple, swelling, oedema, firmness and erythema.

In non-lactating women a subareolar abscess forms often with an inverted or retracted nipple. Mixed growths of anaerobes are usually isolated[16]. Some patients require surgery involving complete duct excision[16]. Abscesses may also be caused by *Pseudomonas aeruginosa* and *Proteus* species[17].

Dental abscess[18-20]

Dental abscesses involve microorganisms colonising the teeth that may become responsible for oral and dental infections, leading to dentoalveolar abscesses and associated diseases. They may also occur as a direct result of trauma or surgery.

Periodontal disease involves the gingiva and underlying connective tissue, and infection may result in gingivitis or periodontitis[21].

Organisms most commonly isolated in acute dentoalveolar abscesses are facultative or strict anaerobes. The most frequently isolated organisms are anaerobic Gram negative rods; however other organisms have also been isolated. Examples include[18,19]:

- α-haemolytic streptococci
- anaerobic Gram negative bacilli
- anaerobic streptococci
- *"S. anginosus"* group
- *Aggregatibacter actinomycetemcomitans*
- *Actinomyces* species
- spirochaetes

Aspiration of dental abscesses may be taken, where possible, to assist in the identification of the causative organism(s). Swabs may be contaminated with superficial commensal flora. In cases of intraosseal abscess, swabs can be useful, but only if taken from a disinfected site.

Liver abscess[22]

Liver abscesses can be amoebic or bacterial (so-called pyogenic) in origin or, more rarely, a combination of the two.

Pyogenic liver abscesses usually present as multiple abscesses and are potentially life-threatening. They require prompt diagnosis and therapy by draining and/or aspirating purulent material, although it is possible to treat liver abscesses with antibiotics alone. They occur in older patients than those with amoebic liver abscesses, and are often secondary to a source of sepsis in the portal venous distribution.

Examples of the sources of pyogenic liver abscess include:

- biliary tract disease

- extrahepatic foci of metastatic infection
- surgery
- trauma

Many different bacteria may be isolated from pyogenic liver abscesses. The most common include[23-25]:

- Enterobacteriaceae
- *Bacteroides* species
- *Clostridium* species
- anaerobic streptococci
- *"S. anginosus"* group
- enterococci
- *P. aeruginosa*
- *B. pseudomallei* (in endemic areas)

Other causes include C*andida* species.

Amoebic liver abscesses arise as a result of the spread of *Entamoeba histolytica* via the portal vein from the large bowel which is the primary site of infection (investigation of amoebae is described in B 31 – Investigation of specimens other than blood for parasites).

Hydatid cysts may also occur as fluid-filled lesions in the liver. However, the clinical presentation is usually different from that of liver abscesses (refer to B 31 – Investigation of specimens other than blood for parasites). Cysts may become super-infected with gut flora and progress to abscess formation.

Lung abscess

Lung abscesses involve the destruction of lung parenchyma and present on chest radiographs as large cavities often exhibiting air-fluid levels. This may be secondary to aspiration pneumonia, in which case the right middle zone is most frequently affected. Other organisms may give rise to multifocal abscess formation and the presence of widespread consolidation containing multiple small abscesses (<2 cm diameter) is sometimes referred to as necrotising pneumonia. Pneumonia caused by *S. aureus* and *Klebsiella pneumoniae* may show this picture (refer to B 57 – Investigation of bronchoalveolar lavage, sputum and associated specimens).

Lung abscesses most often follow aspiration of gastric or nasopharyngeal contents as a consequence of loss of consciousness, resulting for example from alcohol excess, cerebrovascular accident, drug overdose, general anaesthesia, seizure, diabetic coma, or sepsis. Other predisposing factors include oesophageal or neurological disease, tonsillectomy and tooth extraction.

Lung abscesses may arise from endogenous sources of infection. The bacteria involved in these cases are generally from the upper respiratory tract and anaerobes are often implicated, secondarily infecting consolidated lung after aspiration from the upper respiratory tract. Nosocomial infections involving *S. aureus, S. pneumoniae, Klebsiella* species and other organisms may also occur.

B. pseudomallei may cause lung abscesses or necrotising pneumonia in those who have visited endemic areas (mainly South East Asia and Northern Australia) especially in diabetics[26].

Nocardia infection is most often seen in the lung where it may produce an acute, often necrotising, pneumonia[27]. This is commonly associated with cavitation. It may also produce a slowly enlarging pulmonary nodule with pneumonia, associated with empyema. Nocardiosis, almost always occurring in a setting of immunosuppression, may present as pulmonary abscesses.

Actinomyces species cause a thoracic infection that may involve the lungs, pleura, mediastinum or chest wall. Cases often go unrecognised until empyema or a chest wall fistula develops. Aspiration of oral contents is a risk factor for the development of thoracic actinomycosis, thus predisposing conditions include alcoholism, cerebral infarction, drug overdose, general anaesthesia, seizure, diabetic coma or sepsis.

Abscesses as a result of blood borne spread of infection from a distant focus may occur in conditions such as infective endocarditis.

Lemierre's syndrome (or necrobacillosis) originates as an acute oropharyngeal infection usually in a young adult. Infective thrombophlebitis of the internal jugular vein leads to septic embolisation and metastatic infection. The lung is most frequently involved but multifocal abscesses may develop. *Fusobacterium necrophorum* is the most common pathogen isolated from blood cultures in patients with this syndrome[28].

Aspergillus species have been isolated from lung abscesses in patients who are immunocompromised.

Pancreatic abscess

Pancreatic abscesses are potential complications of acute pancreatitis. Infections are often polymicrobial and common isolates include *Escherichia coli*, other Enterobacteriaceae, enterococci and anaerobes; longer-standing collections, especially after prolonged antibiotic therapy, are often infected with coagulase negative staphylococci and *Candida* species[29].

Perirectal abscess

Perirectal abscesses are encountered in patients with predisposing factors. These include[30]:

- immunodeficiency
- malignancy
- rectal surgery
- ulcerative colitis

They are often caused by[31]:

- anaerobes
- Enterobacteriaceae
- streptococci
- *S. aureus*

Pilonidal abscess

Pilonidal abscesses result from infection of a pilonidal sinus. Anaerobes and Enterobacteriaceae are usually isolated, but they may be caused by *S. aureus* and β-haemolytic streptococci[32].

Prostatic abscess[33]

Abscesses within the prostate may be caused by, or associated with[34]:

- diabetes mellitus
- acute and chronic prostatitis
- instrumentation of the urethra and bladder
- lower urinary tract obstruction
- haematogenous spread of infection

Organisms that may cause prostatic abscesses include[35]:

- *E. coli* and other Enterobacteriaceae
- enterococci
- anaerobes
- *Neisseria gonorrhoea*
- *S. aureus*[33]

Prostatic abscesses can act as reservoirs for *Cryptococcus neoformans* resulting in relapses of infection with this organism[36].

Psoas abscess

Psoas abscesses may be seen as secondary infections to:

- appendicitis
- diverticulitis
- osteomyelitis of the spine
- infection of a disc space
- bacteraemia
- perinephric abscess

Pus tracks under the sheath of the psoas muscle. Infection often occurs in drug abusers after injection into the ipsilateral femoral vein.

Psoas abscesses are predominantly caused by[37,38]:

- Enterobacteriaceae
- *Bacteroides* species
- *S. aureus*
- streptococci
- *Mycobacterium tuberculosis*

Renal abscess

Renal abscesses are typically caused by Gram negative bacilli and result from ascending urinary tract infection, pyelonephritis, renal calculi or sepsis[39].

Renal abscesses are localised in the renal cortex and may occur as a result of, for example, *Staphylococcus aureus* bacteraemia. Pyuria may also be present, but urine culture is usually negative. Renal abscesses are increasingly being seen as complications of acute pyelonephritis caused by Gram negative bacilli. The rare condition of emphysematous pyelonephritis, which results in multifocal intrarenal abscesses and gas formation within the renal parenchyma, is usually seen in diabetic patients or as a complication of renal stones. The commonest cause is *Escherichia coli* and the condition carries a 70% mortality rate.

Perinephric abscess are an uncommon complication of UTI, which usually affects patients with one or more anatomical or physiological abnormalities[40]. The abscess may be confined to the perinephric space or extend into adjacent structures. Pyuria, with or without positive culture, is normally, but not invariably seen on examination of urine. Causative organisms are usually Gram negative bacilli, but can also be staphylococci or *Candida* species. Mixed infections have also been reported.

Salivary gland abscess

There are three pairs of major salivary glands; the parotid, submandibular and sublingual. Parotic abscesses are more commonly seen in the elderly. Common organisms include:

- *S. aureus*
- anaerobes

Spinal epidural abscess[41]

Spinal epidural abscesses may occur in patients with:

- predisposing disease (such as diabetes)
- prior infection elsewhere in the body which may serve as a source for haematogenous spread
- abnormality of, or trauma to, the spinal column (often involving invasive medical procedures such as epidural catheterisation)

The most common isolate is *S. aureus*[42]. *Staphylococcus epidermidis* may be isolated in patients following invasive spinal manipulation. Streptococci (α-haemolytic, β-haemolytic and *S. pneumoniae*), Enterobacteriaceae and pseudomonads may also be isolated[42,43].

Subphrenic abscess

Subphrenic abscesses occur immediately below the diaphragm, often as a result of[44]:

- gastric, duodenal or colonic perforation
- acute cholecystitis
- procedures on the liver and upper part of the gastrointestinal tract
- ruptured appendix

- trauma

Subphrenic abscesses are caused by mixed infections from the normal gastrointestinal flora[44].

Throat/neck abscess

Throat and neck abscess are relatively common[45-47].

Causative organisms include[45,48]:

- β-haemolytic streptococci
- anaerobes

Surgical incision and drainage may be undertaken through intraoral or extra oral procedures.

Unusual cases of abscess formation

Unusual cases of abscess formation can occur in patients with many underlying conditions and may be caused by a vast range of organisms[49-56]. Any organism isolated from abscess pus is potentially significant.

Actinomycosis is a chronic suppurative infection characterised by chronic abscess formation with surrounding fibrosis. It is rare and usually follows perforation of a viscous, trauma or surgery. It is caused by *Actinomyces israelii*, usually in mixed culture with other bacteria[57].

Abscess formation is most often associated with the gastrointestinal tract, the jaw and the pelvis. Other areas of the body may be involved and the formation of abdominal abscesses may occur. Thoracic involvement occurs in 15% of cases of actinomycosis. Pulmonary actinomycosis can be difficult to diagnose prior to cutaneous involvement, which results in direct extension through the chest wall. The disease progresses to form a chronic indurated mass with draining fistulae. Material should be drained from abscesses and biopsies taken. Skin biopsies may reveal the presence of organisms (refer to B 17 – Investigation of tissues and biopsies from deep-seated sites and organs).

"Sulphur granules" are sought in the pus specimen[58]. These are discharged from actinomycosis abscesses. Sulphur granules are colonies of organisms forming a filamentous inner mass which is surrounded by host reaction. They are formed only *in vivo*. They are hard, buff to yellow in colour, and have a clubbed surface.

Intra-abdominal sepsis

Intra-abdominal sepsis is infection occurring in the normally sterile peritoneal cavity[59]. The term covers primary and secondary peritonitis, as well as intra-abdominal abscesses.

Primary peritonitis is infection of the peritoneal fluid in which no perforation of a viscous has occurred. Infection usually arises via haematogenous spread from an extra-abdominal source and is often caused by a single pathogen[59]. It is common in patients with ascites following hepatic failure. In females it may also result from organisms ascending the genital tract (refer to B 28 - Investigation of genital tract associated specimens).

Secondary peritonitis is acute, suppurative inflammation of the peritoneal cavity usually resulting from bowel perforation or postoperative gastrointestinal leakage. Secondary peritonitis is most often treated with a combination of drainage and antibiotics.

The most frequent isolates encountered in intra-abdominal sepsis with secondary peritonitis are derived from the normal gastrointestinal flora. Anaerobic bacteria are isolated from the majority of cases with *Bacteroides* species being isolated. However, infections are usually polymicrobial and organisms that have been isolated include:

- *Enterococcus* species
- *Bacteroides* species
- pseudomonads
- *Peptostreptococcus* species
- yeasts (mostly *Candida* species)
- β-haemolytic streptococci
- *Clostridium* species
- Enterobacteriaceae

Tuberculous peritonitis is a rare disease in the UK. It is more common on the Indian sub-continent, so it is important to consider this in immigrants from that area. In most cases a primary pulmonary focus is present with secondary spread of *Mycobacterium tuberculosis* (refer to B 40 – Investigation of specimens for *Mycobacterium* species).

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[60,61]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk

of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Rapid methods

To reduce turnaround times, rapid identification and sensitivity tests may be performed in conjunction with routine methods where appropriate. A variety of rapid identification and sensitivity methods have been evaluated; these include molecular techniques and the Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[62,63]. It is important to ensure that fresh cultures of pure single isolates are tested to avoid reporting misleading results.

Laboratories should follow manufacturers' instructions and all rapid tests must be validated and be shown to be fit for purpose prior to use.

1 Safety considerations[60,61,64-78]

1.1 Specimen collection, transport and storage[60,61,64-67]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Avoid accidental injury when pus is aspirated.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[60,61,64-78]

ContGram positiveainment Level 2.

If infection with a Hazard Group 3 organism eg *Mycobacterium* species, *Paracoccidioides brasiliensis* or *Brucella* species is suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions. Thus initial examination and all follow up work on specimens from patients with suspected *Mycobacterium* species, or suggesting a diagnosis of blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosis or penicilliosis must be performed inside a microbiological safety cabinet under full Containment Level 3 conditions.

It is recommended that all Gram negative coccobacilli from sterile sites should be processed in a Class I or Class II microbiological safety cabinet until Hazard Group 3 pathogens (ie Brucella) have been definitively excluded[79].

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[70].

Any grinding of sulphur granules should be performed in a microbiological safety cabinet.

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65-75°C), under the hood, until dry. Then place in a rack or other suitable holder.

Note: Heat-fixing may not kill all *Mycobacterium* species[80]. Slides should be handled carefully.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Pus, exudate

2.2 Optimal time and method of collection[81]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[81].

Samples of pus are preferred to swabs. However, pus swabs are often received. When using a swab disinfect the superficial areas first. The deepest part of the wound should be sampled, avoiding the superficial microflora.

The specimen will usually be collected by a medical practitioner. Cleaning the site with sterile saline or 70% alcohol is recommended by some sources[82].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[83-87].

2.3 Adequate quantity and appropriate number of specimens[81]

Ideally, a minimum volume of 1mL of pus should be submitted.

Swabs are not the optimal sample type. However, if received, swabs should be well soaked in pus. Refer to B 11 - Investigation of swabs from skin and superficial soft tissue infections.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport and storage[60,61]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[81].

The volume of specimen influences the transport time that is acceptable. Large volumes of purulent material maintain the viability of anaerobes for longer[88,89].

The recovery of anaerobes in particular is compromised if the transport time is delayed.

If processing is delayed, refrigeration is preferable to storage at ambient temperature.

4 Specimen processing/procedure[60,61]

4.1 Test selection

Divide specimen on receipt for appropriate procedures such as examination for parasites (B 31 – Investigation of specimens other than blood for parasites) and culture for *Mycobacterium* species (B 40 – Investigation of specimens for *Mycobacterium* species), depending on clinical details.

4.2 Appearance

Describe presence or absence of sulphur granules (if sought).

4.3 Sample preparation

4.3.1 Pre-treatment

Exudates

Centrifuge in a sterile, capped, conical-bottomed container at 1200 x g for 5-10 min.

Transfer the supernatant with a sterile pipette, leaving approximately 0.5mL, to another CE marked leak proof container in a sealed plastic bag for additional testing if required.

Resuspend the deposit in the remaining fluid.

Supplementary

Wash any sulphur granules that are present in saline.

Suspend an aliquot of pus containing sulphur granules in sterile water or saline in a CE marked leak proof container in a sealed plastic bag. Agitate gently to wash pus from the granules.

Grind the washed granules in a small amount of sterile water or saline, with a sterile tissue grinder (Griffiths tube or unbreakable alternative) or a pestle and mortar.

Use this homogenised sample to make a smear for Gram staining and to inoculate agar plates.

Note 1: All grinding of sulphur granules should be performed in a microbiological safety cabinet.

Note 2: If a fungal infection is suspected then grinding of the whole specimen should be avoided. This is to prevent damaging hyphae that would result in a reduced yield, particularly with zygomycetes.

4.3.2 Specimen processing

Pus

Inoculate agar plates and enrichment broth with the pus or centrifuged deposit with a sterile pipette (refer to Q 5 – Inoculation of culture media for bacteriology).

If sulphur granules are present, these should be ground and included in the culture.

For the isolation of individual colonies, spread inoculum with a sterile loop.

All additional pus/fluid from the specimen should be stored at 4°C for at least 7 days after the issue of the final report.

Swabs

If a swab of pus is received, follow the recommendations in B 11 – Investigation of swabs from skin and superficial soft tissue infections.

4.4 Microscopy

4.4.1 Standard

Swab

Prepare a thin smear on a clean microscope slide for Gram staining after performing culture (refer to Q 5 – Inoculation of culture media for bacteriology).

Pus

Using a sterile pipette place one drop of neat specimen or centrifuged deposit (see 4.5.1), as applicable, on to a clean microscope slide. Spread this using a sterile loop to make a thin smear for Gram staining (refer to TP 39 – Staining procedures). The Gram film result should be used as a guide for supplementary cultures (eg fungal, Actinomyces) when appropriate.

4.4.2 Supplementary

Gram stain of sulphur granules

With care, either squash the sulphur granules that have been washed in saline between two slides using gentle pressure, or use the homogenised granules (see section 4.5.1) and make a thin smear for Gram staining.

Note: Any grinding of sulphur granules should be performed in a microbiological safety cabinet.

For microscopy, *Mycobacterium* species (B 40 – Investigation of specimens for *Mycobacterium* species) and parasites (B 31 – Investigation of specimens other than blood for parasites). For fungi and other staining procedures refer to TP 39 – Staining procedures.

4.5 Culture and investigation

Inoculate each agar plate using a sterile pipette (Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
All clinical conditions	All pus and exudates	Blood agar	35-37	5–10% CO_2	40-48hr	daily	Any organism
		CLED/ MacConkey agar	35-37	Air	18-24hr	≥18hr	
		Selective anaerobe agar with a metronidazole 5µg disc	35-37	Anaerobic	5 d	≥40hr and at 5 d	Anaerobes
		Fastidious anaerobic cooked meat broth or equivalent.	35-37	Air	5 d	N/A	Any organism
		Subculture if evidence of growth (≥40hr), or at day 5 to above media	35-37	As above	40-48hr	≥40hr	

		(excluding MacConkey)					
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Submandibular abscess Brain abscess Liver abscess Lung empyema/ abscess Psoas abscess Spinal abscess	Pus	Chocolate agar	35–37	5–10% CO_2	40 – 48hr	≥40hr	Fastidious organisms
Actinomycosis (or where microscopy suggestive of actinomycetes)	Pus	Blood agar supplemented with metronidazole and nalidixic acid	35-37	Anaerobic	10 d	≥40hr, at 7 d and 10 d	*Actinomyces* species
Nocardiosis	Pus	Blood agar	35-37	5-10% CO_2	16-48hr	daily	*Nocardia* species*
Immunocompromised or fungi suspected (Gram film or clinical details)	Pus	Sabouraud agar	28-30	Air	14 d	daily	Yeast Mould
Prostatic abscess Primary peritonitis in females	Pus	GC selective/ Chocolate agar	35-37	5-10% CO_2	40-48hr	≥40hr	*N. gonorrhoeae*
Clinical details/ conditions	**Specimen**	**Optional media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
When clinical details or microscopy suggestive of mixed infection	Pus	Staph/strep selective agar	35-37	Air	40-48hr	daily	*S. aureus* Streptococci
		Selective Gram negative anaerobe medium	35-37	Anaerobic	Up to 5 d	≥40hr and 5 d	Gram negative anaerobes

Other organisms for consideration - Fungi (B 39 – Investigation of dermatological specimens for superficial mycoses) and *Mycobacterium* species (B 40 – Investigation of specimens for *Mycobacterium* species) *Refer to B 17 – Investigation of tissues and biopsies from deep-seated sites and organs) for isolation of *Nocardia* species using specimen type, biopsy (this includes bronchoalveolar lavage (BAL)).

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Actinomycetes	species level ID 10 – Identification of aerobic Actinomycetes ID 15 – Identification of anaerobic *Actinomyces* species
Anaerobes	genus level (in brain samples to species level)
ß-haemolytic streptococci	species level
Coagulase negative staphylococci	"coagulase negative" level
Enterobacteriaceae	genus level
Yeast	species level
Mould	species level
Neisseria	species level
Pseudomonads	species level
S. aureus	species level (consider Panton-Valentine leukocidin (PVL) and toxin testing if appropriate clinical details) (consider toxin testing on samples from post mortem samples)
"*S. anginosus*" group	"*S. anginosus*" group level
Mycobacterium	B 40 - Investigation of specimens for *Mycobacterium* species
Parasites	B 31 - Investigation of specimens other than blood for parasites

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC), EUCAST and/or CSLI guidelines or manufacturer's validation for preparatory methods.

This SMI does not contain recommendations for the selective and restrictive reporting of susceptibilities to antimicrobials due to the diversity of organisms associated with pus and exudate samples. Local decisions on antimicrobial susceptibility testing should be subject to consultation that should include local antimicrobial stewardship groups.

4.7.1 Antimicrobial susceptibility testing and reporting table

N/A

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Consider sending *S. aureus* isolates for toxin testing where appropriate clinical details are provided. For example, isolates from post mortems where the specimen is suspected to be the cause of death should be sent for toxin testing.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Gram stain

Report on WBCs and organisms detected.

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

For the reporting of microscopy for fungi, *Mycobacterium* species and parasites (B 40 – Investigation of specimens for *Mycobacterium* species) and parasites (B 31 – Investigation of specimens other than blood for parasites).

5.2 Culture

The following results should be reported:

- clinically significant organisms isolated
- other growth

- absence of growth

Report on the presence of sulphur granules.

Also, report results of supplementary investigations: fungi, *Mycobacterium* species and parasites. (B 31 – Investigation of specimens other than blood for parasites).

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated; guidance on selective reporting is not included in this SMI. Prudent use of antimicrobials according to local and national protocols is recommended.

Generally, all resistant results should be reported as this is good practice and informs the user.

6 Notification to PHE[90,91], or equivalent in the devolved administrations[92-95]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

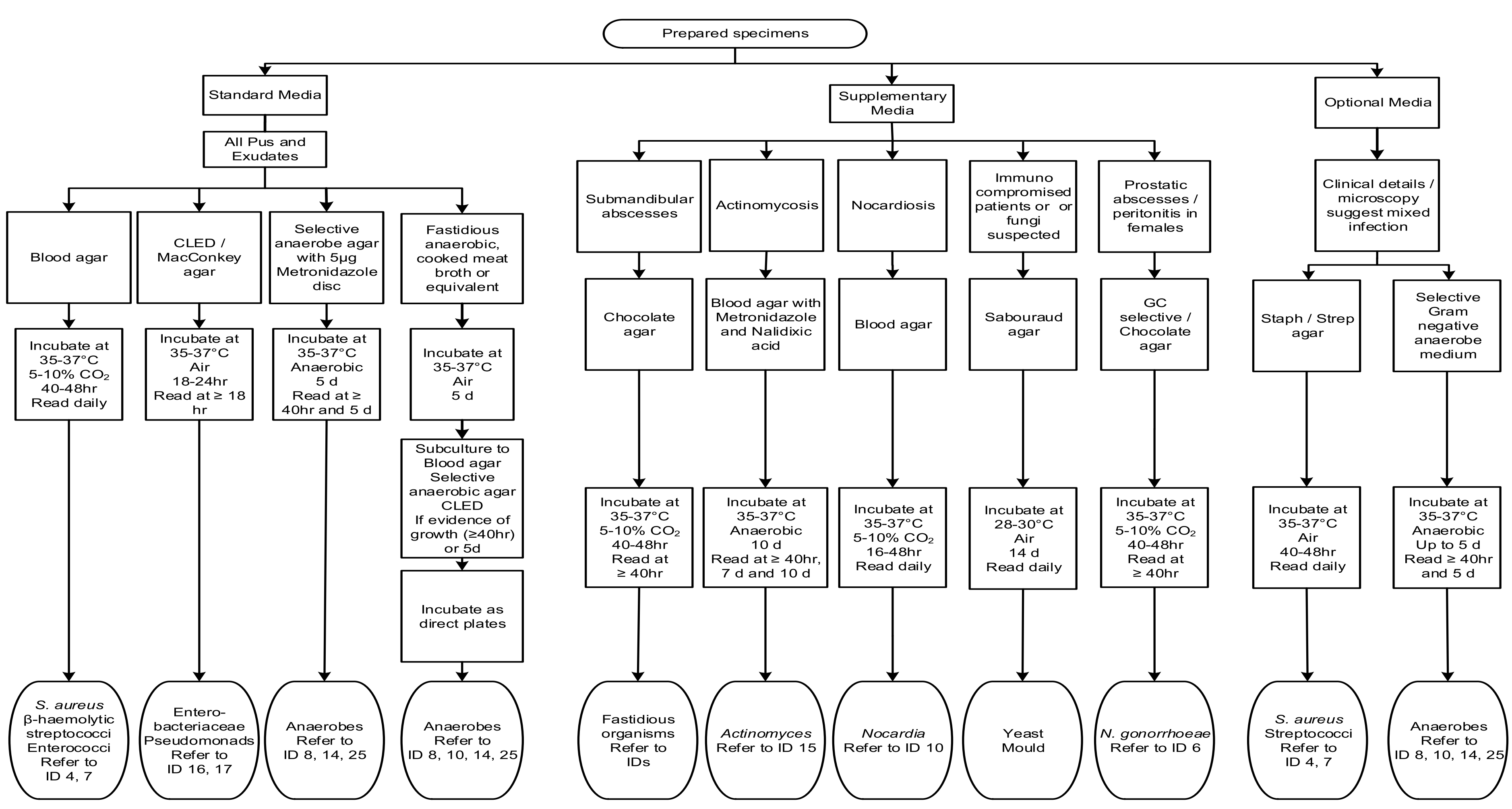
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[92,93], Wales[94] and Northern Ireland[95].

Appendix: Investigation of pus and exudates[96]



UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England

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UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

B 14 Investigation of pus and exudates

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 06/01/2015 – 26/01/2015

Version of document consulted on: B 14dk+

Proposal for changes

Comment number	1		
Date received	13/01/2015	**Lab name**	Microbiology, CPL, St James's hospital, Dublin, Ireland
Section			
Comment			
Actinobacillus actinomycetemcomitans changing its name to aggregatibacter actinomycetemcomitans.			
Evidence			
Websites with information on HACEK.			
Financial barriers			
No.			
Recommended action	**ACCEPT** Text updated.		

Comment number	2		
Date received	15/01/2015	**Lab name**	Nottingham University Hospitals
Section	4.6.1 minimum level of identification		
Comment			
Enterobacteriaceae identification to 'coliform' level only-for these sterile site specimens I would have thought to direct likely source investigation and antibiotic management this should be to at least genus level.			
Recommended action	**ACCEPT** Text updated.		

Comment number	3		
Date received	16/01/2015	Lab name	PathWest Pathology
Section			
Comment			
Cleaning the site with sterile saline or 70% alcohol is recommended in some sources.			
Evidence			
Versalovic, James, and American Society for Microbiology 2011 Manual of Clinical Microbiology. Washington, DC: ASM Press.			
Financial barriers			
No.			
Recommended action	ACCEPT The statement has been added and appropriate journal references sought.		

Comment number	4		
Date received	21/01/2015	Lab name	Northern Health and Social Care Trust
Section	Introduction		
Comment			
Under Throat abscess - Add text from reference 55 not sure if that is a proofing error or if it was purposely left that way.			
Financial barriers			
Space problems in anaerobic cabinet.			
Health benefits			
No.			
Recommended action	ACCEPT Text amended.		

Comment number	5		
Date received	23/01/2015	**Lab name**	Truro
Section	Pages 20 and 24		
Comment			
Staph/strep selective agar incubation time, we read at 24 hours.			
Recommended Action	**NONE** The group agreed that the incubation period of 48hr (reading daily) was optimal.		

Comment number	6		
Date received	26/01/2015	**Lab name**	IBMS
Section	a. Introduction – Dental Abscess b. Whole document c. Introduction – Throat Abscess d. Page 11 e. Section 1.2 f. Section 4.6.1 g. Section 4.6.1 h. Section 1.2 i. Section 1.2 j. Technical Information/Limitations – Specimen Containers k. Section 4.7		
Comment			
a. Dental abscess section. Actinobacillus actinomycetemcomitans has been reclassified as Aggregatibacter actinomycetemcomitans. (See attached paper) b. Bacterial names need to be italicised throughout. A few have been missed in the Renal abscess section. c. Throat abscess section 'Throat abscess are relatively common. Add text from reference' –text from the reference must be added. d. Page 11- Correction of nomenclature required Penicillium marneffi is now Talaromyces marneffei e. Specimen processing section 1.2 'It is recommended that all Gram-negative coccobacilli from (TEXT MISSING HERE) should be processed in a Class I or Class II microbiological safety cabinet until			

<table>
<tr><td colspan="2">

Hazard Group 3 pathogens (ie Brucella) have been definitively excluded.' Same text is missing as in B17.

f. Line 9 Confusion with regards the identification of yeast isolates. ?what is yeast to yeast level? Is this calling the organism "yeast" rather than identifying it?

g. Fungi species level (except yeast to yeast level)

h. Typo Line 12 "Paracoccoides brasiliensis or Brucella species is suspected, all specimens must be" Paracoccidioides brasiliensis

i. Typo Line 19 some of the sentence is missing "It is recommended that all Gram-negative coccobacilli from *** should be processed in a"

j. Under the specimen containers section it mentions that CE marked leak proof containers should be used, but there is no reference to M40 complaint swabs (B11 and B14 only) despite stating that samples on swabs were acceptable for investigation. The CLSI M40-A2 Quality Control of Microbiological Transport Systems was revised in June 2014 and is the expected standard for transport swabs.

k. Under the antimicrobial susceptibility testing each document make reference to BSAC or EUCAST which is fine for bacterial pathogens. However, for Candida and Moulds (which are mentioned in the text) only CLSI breakpoints apply.

</td></tr>
<tr><td>Evidence</td><td>a. http://ijs.sgmjournals.org/content/56/9/2135.long</td></tr>
<tr><td>Recommended action</td><td>

a. **ACCEPT**

b. **ACCEPT**

c. **ACCEPT**

Text added.

d. **ACCEPT**

e. **ACCEPT**

Missing text replaced with 'sterile sites'.

f. **ACCEPT**

It was agreed that 'yeast to yeast level' should be replaced with 'species' level.

g. **PARTIAL ACCEPT**

It was agreed that 'yeast to yeast level' would be removed.

h. **ACCEPT**

i. **ACCEPT**

j. **NONE**

It was agreed that this was outside of the scope of the document.

k. **ACCEPT**

A reference to CSLI breakpoints will be made for Mould.

</td></tr>
</table>

Comments received outside of consultation

Comment number	1		
Date received	02/02/2015	**Professional body**	ACOM
Section	Various		

Comment

a. Brain Abscess

Add 'or bacteraemia' to second bullet point.

b. Dental Abscess

Change text to read 'Periodontal diseases involve the gingiva (gingivitis) and underlying connective tissue including bone (periodontitis)'.

c. Dental Abscess

Anaerobic Gram negative bacilli: Fusobacteria would deserve a mention as they are often involved with metastatic spread (liver/brain abscesses).

d. Dental Abscess

Staphylococci: Reference for example Gronholm et al 2012: Interestingly, staphylococci were reported in over 30 % of the pus samples and S. aureus in 9 % of the samples.

e. Dental Abscess

Add coliforms to bullet points.

f. Dental Abscess

Spirochaetes: Moved to the end of the bullet points as they cannot be cultured.

g. Dental Abscess

'Aspiration of dental abscesses is necessary to obtain samples containing the likely causative organisms. Swabs are likely to be contaminated with superficial commensal flora.' - May be true but intraosseal abscess aspiration is nearly impossible and swabbing the incision site pus (if disinfected prior to incision) is a decent sample.

h. Dental Abscess

Add: 'In case of intraosseal abscess, swabs can be useful but only if taken from a disinfected incision site.'

i. Intra-abdominal sepsis

Yeasts: Mostly Candida spp.

j. Section 1.2

Maybe needs to be expanded to clarify when to suspect these (travel history, sampling site).

k. Section 2.2

However, pus swabs are often received (when using swabs, the deepest part of the wound should be sampled ***after disinfecting the superficial areas first***, avoiding the superficial microflora).

l. Section 4.6.1 Anaerobes level seems to stand out - almost everything else is species level. Would be useful to identify at least to genus level (or even as grampus/ned and coccus/rod). m. Section 4.6.1 Fungi - species level: This is a big ask! This means sending all moulds to reference laboratory. But it would be clinically very useful, so leave as it is. n. Section 4.6.1 Fungi - except yeast to yeast level: Is this relevant today - most labs can easily identify yeasts to genus level, even species level. Depending on the sample would not be a big ask to identify to species (or at least to genus) level.	
Evidence	
Gronholm et al 2012 The role of unfinished root canal treatment in odontogenic maxillofacial infections requiring hospital care.	
Recommended action	a. **NONE** It was felt that this was sufficiently covered by the current text. b. **ACCEPT** Text updated. c. **NONE** The inclusion of Fusobacterium was discussed and it was agreed that the inclusion of anaerobic Gram negative bacilli in the list of causative organisms was sufficient. d. **NONE** For information, no action required. e. **NONE** The group felt that it was not necessary to include Coliforms in the list of organisms for dental abscesses. f. **ACCEPT** Text updated. g. **ACCEPT** Text update to: Aspiration of dental abscesses may be taken (where possible) to assist in the identification of the causative organism(s). Swabs may be contaminated with superficial commensal flora. h. **ACCEPT** Text included. i. **ACCEPT** Text updated. j. **NONE**

	It was felt that section 1.2 was sufficiently detailed. k. **ACCEPT** Text updated. l. **ACCEPT** Text updated to genus level. m. **NONE** No action required. n. **ACCEPT** Yeast and moulds identified to species level.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	06/01/2015	**Lab name**	Microbiology Queen Elizabeth Hospital LGHT SE18 4QH

UK Standards for Microbiology Investigations

Investigation of bile

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Issued by the Standards Unit, National Infection Service, PHE

Bacteriology | B 15 | Issue no: 7 | Issue date: 11.01.18 | Page: 1 of 21

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Logos correct at time of publishing.

Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 5

Scope of document 8

Introduction 8

Technical information/limitations 10

1 Safety considerations 11

2 Specimen collection 11

3 Specimen transport, storage and retention 12

4 Specimen processing/procedure 12

5 Reporting procedure 15

6 Notification to PHE, or equivalent in the devolved administrations 15

Appendix: Investigation of bile 17

References 18

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	10/11.01.18
Issue number discarded	6
Insert issue number	7
Anticipated next review date*	11.01.21
Section(s) involved	**Amendment**
Whole document.	Document was reviewed with minor amendments and references updates.

*Reviews can be extended up to five years subject to resources available.

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UK SMI#: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (2018). Investigation of bile. UK Standards for Microbiology Investigations. B 15 Issue 7. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Bile

This UK SMI describes the processing and bacteriological investigation of bile.

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

Biliary infection can produce significant morbidity and mortality and the prognosis often depends upon whether biliary tract obstruction is present. Gram negative bacteria (mainly *Escherichia coli*) are the cause of the majority of biliary infections although Gram positive and anaerobic organisms are also found[1,2]. Biliary infection presents as either cholangitis or cholecystitis.

Bile is normally sterile, however colonisation may occur, frequently with a mixture of aerobes and anaerobes originating from the gut[3]. Occasionally instrumentation or stenting may lead to colonisation or infection, which may progress to bacteraemia[4]. Fever, previous endoscopic or percutaneous biliary instrumentation, and bilioenteric anastomosis are significant predictors of a positive bile culture[2].

Cholangitis

Cholangitis is the inflammation of the biliary ducts. It may present in two forms, ascending or suppurative cholangitis[3].

Ascending cholangitis

Ascending cholangitis occurs when partial obstruction of the biliary ducts and bacterial proliferation in the bile occur together[3,5]. Bacteria are shed intermittently into the bloodstream. This can develop into suppurative cholangitis. Ascending cholangitis is a common cause of sepsis following liver transplantation.

Suppurative cholangitis

Suppurative cholangitis occurs when an infected biliary system is completely obstructed. Biliary pressure increases and bacteria are constantly shed into the bloodstream. Diagnosis of infection can be made by aspirating bile and taking blood cultures (B 37 - Investigation of blood cultures (for organisms other than *Mycobacterium* species)).

Recurrent pyogenic cholangitis

Recurrent pyogenic cholangitis presents as episodes of right abdominal pain, biliary obstruction and cholangitis and Gram negative septicaemia in patients that are chronically infected with biliary parasites.

Cholecystitis

Cholecystitis is inflammation of the gall bladder. It is usually due to an infection that is often secondary to the presence of gallstones. When the cystic duct is obstructed by a gallstone the hydrostatic pressure in the gallbladder lumen is increased. This produces pain and infection frequently ensues.

Emphysematous cholecystitis

Emphysematous cholecystitis is an acute infective cholecystitis involving gas-forming organisms, most commonly *Clostridium perfringens*. Gangrene and perforation may result.

Endoscopic retrograde cholangiopancreatography (ERCP)

One of a variety of imaging techniques used to study the biliary tree, whereby an endoscope is passed from the gut via the ampulla of Vater into the biliary ducts. This is minimally invasive but may cause biliary sepsis.

Organisms isolated from bile include[3,5]:

- Enterobacteriaceae
- *Enterococcus* species
- Pseudomonads
- *Bacteroides* species
- *Clostridium* species
- Anaerobes
- *Staphylococcus aureus*
- *Salmonella*

Other organisms may be isolated and should be given consideration depending on clinical details.

Yeast infections

Yeast infections are rare in normal individuals. They occur in older patients with malignancy, immunocompromised patients, diabetic patients or in patients receiving antimicrobial treatment for other infections. Such infections may be confined to the biliary tract or be a feature of more general candidosis. They usually involve *Candida albicans*, but other *Candida* species have been reported[2,6-8].

Parasitic invasion

Parasitic invasion of the biliary tract occurs in patients from or in the developing world or those who are immunosuppressed and may involve[5]:

- *Ascaris lumbricoides*
- *Clonorchis sinensis*
- *Opisthorchis* species
- *Fasciola hepatica*
- *Giardia intestinalis*
- *Cryptosporidium* species
- Microspora

These are described in B 31 - Investigation of specimens other than blood for parasites.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[9,10]

UK SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

1 Safety considerations[9-25]

1.1 Specimen collection, transport and storage[9-14]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[9-25]

Containment Level 2.

As a minimum, it is recommended that the processing of any culture that may result in generation of aerosols should be processed in a microbiological safety cabinet in accordance with the relevant risk assessment, ACDP and HSE guidelines[17].

Processing of diagnostic sample cultures that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under appropriate containment conditions as determined by risk assessment, and as required by Biological agents: managing the risks in laboratories and healthcare premises[17]. This will normally be under full CL3 conditions. Such organisms include Mycobacterium species, Brucella species, Bacillus anthracis, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidiodes immitis, etc.

Diagnostic work with clinical material that could possibly contain Hazard Group 3 organisms (*Salmonella* Typhi and *Salmonella* Paratyphi A,B & C,) does not normally require full Containment Level 3 containment (paragraph 175)[17].

Note: *S*. Typhi and *S.* Paratyphi A, B and C cause severe and sometimes fatal disease and laboratory acquired infections have been reported. *S.* Typhi vaccination is available. Guidance is given in the Public Health England immunisation policy.

2 Specimen collection

2.1 Type of specimens

Bile

2.2 Optimal time and method of collection[26]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[26].

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[27-31].

Bile may be collected in theatre or from a closed drainage system by aspiration with a needle and syringe.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens[26]

Ideally, a minimum volume of 1mL.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport, storage and retention[9,10]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[26].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[26].

The volume of specimen influences the viability of anaerobes[32-34].

The recovery of anaerobes is compromised if the transport time exceeds 3hr[34].

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'[35].

4 Specimen processing/procedure[9,10]

4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as examination for parasites (B 31 - Investigation of specimens other than blood for parasites) depending on clinical details.

4.2 Appearance

The presence of pus should be noted.

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Using a sterile pipette place one drop of specimen on to a clean microscope slide.

4.4.2 Supplementary

Microscopy for parasites – see B 31 - Investigation of specimens other than blood for parasites.

If a Gram stain is required, spread one drop of the specimen with a sterile loop to make a thin smear on a clean microscope slide.

4.5 Culture and investigation

Using a sterile pipette inoculate each agar plate and enrichment broth, if included, with specimen (see Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Cholangitis Cholecystitis	Bile	Blood agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
		CLED*/ MacConkey agar	35-37	air	16-24hr	≥16hr	
		Neomycin fastidious anaerobe agar	35-37	anaerobic	40-48hr **	≥48hr***	Anaerobes
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Salmonella carriage/infection	Bile	Mannitol selenite F broth then subcultured to XLD	35-37 35-37	air air	16-24hr 16-24hr	N/A ≥16hr	*Salmonella* species

* CLED agar, originally designed for urine specimens

** Prolonged 14-day incubation might be of interest in particular situations in which the prevalence of slow-growing microorganisms and anaerobes is higher; in such cases plates should be left in the incubator/cabinet, read at 5 days and then again left in the incubator/cabinet until day 14[36]

*** if the laboratory has an anaerobic cabinet plates may be read at 48 hours, ideally they should be left for 5 to 7 days

4.6 Identification

Refer to individual UK SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Note: All work on *S.* Typhi and *S.* Paratyphi A, B & C must be performed in a microbiological safety cabinet in a Containment Level 3 room.

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
Enterobacteriaceae (not *Salmonella* species)	"coliforms" level
Enterococci	genus level
P. aeruginosa	species level
Other *Pseudomonas*	"pseudomonas" level
Salmonella	*S.* Typhi, *S.* Paratyphi or other serogroup level Whole genome sequencing[37]
S. aureus	species level

Streptococci	genus or Lancefield group level
C. albicans	species level
Other *Candida* species	genus level
Parasites	see B 31 - Investigation of specimens other than blood for parasites

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to EUCAST guidelines for breakpoints. Additional UK specific susceptibility testing guidance is available on British Society for Antimicrobial Chemotherapy (BSAC) webpage.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

β-haemolytic streptococci	Serotyping
S. aureus	Spa typing
Salmonella	Serotyping and phage typing (if applicable)
Fungi	Identification and/or susceptibility testing

5 Reporting procedure

5.1 Microscopy

Report the WBCs and organisms detected.

Microscopy for parasites – see B 31 - Investigation of specimens other than blood for parasites.

5.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report 16–72hr.

5.2 Culture

Report clinically significant organisms isolated (with an appropriate comment on possible contamination or overgrowth if the specimen is from a collection bag or T-tube) or

Report: other growth or absence of growth.

Also, report results of supplementary investigations.

Culture reporting time.

Clinically urgent results to be telephoned or sent electronically.

Written report, 16 – 72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations: Parasites – see B 31 - Investigation of specimens other than blood for parasites.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[38,39], or equivalent in the devolved administrations[40-43]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many

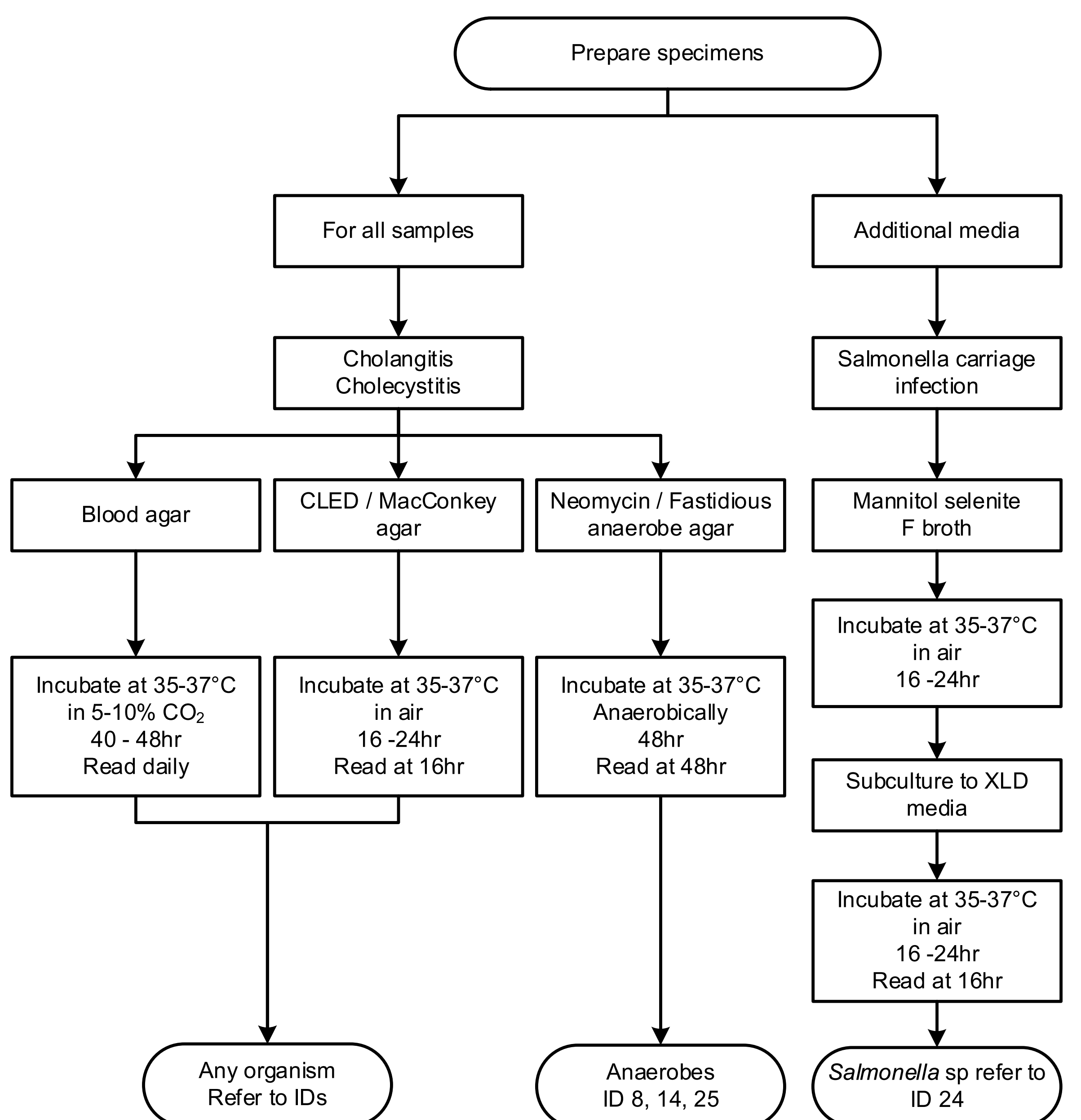
PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[40,41], Wales[42] and Northern Ireland[43].

Appendix: Investigation of bile



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References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

B 15 Investigation of bile

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 11/08/2017 – 25/08/2017

Version of document consulted on: B 6dc+

Proposal for changes

Comment number	1		
Date received	17/08/2017	**Lab name**	Keith Shuttleworth and Associates Ltd
Section	1 1:1-Specimen Collection Transport and Storage		
This UK SMI B 15 describes the processing and bacteriological investigation of bile. Considering that the processing of bile samples is very similar to the processing of other sterile fluids, the Standards Unit is considering merging the UK SMI B 15 with the UK SMI B 26 on the investigation of fluids from normally sterile sites. Would you agree with this merging? Please comment.			
I am not sure.			
Recommended action	**NONE**		
General comment			
I was only thinking that if there were some pictures/posters of the type of CE Marked leak proof containers at the last page of this document, it would make things really easy for staff, in terms of purchasing the appropriate containers, saving unnecessary waste of money buying the wrong thing. Poster could also be displayed at work places.			
Evidence			
I have evidenced the use of similar posters/ pictures displayed at work places for collection of sensitive specimens.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**NONE** Currently not part of the process.		

Comment number	2		
Date received	21/08/2017	**Lab name**	States of Jersey Pathology Laboratory
Section	Introduction (parasite list)		
This UK SMI B 15 describes the processing and bacteriological investigation of			

bile. Considering that the processing of bile samples is very similar to the processing of other sterile fluids, the Standards Unit is considering merging the UK SMI B 15 with the UK SMI B 26 on the investigation of fluids from normally sterile sites. Would you agree with this merging? Please comment.	
Yes.	
Recommended action	**ACCEPT**
General comment	
Giardia lamblia is listed. We have now altered our taxonomy used to *Giardia intestinalis*. Would you still use *G. lamblia* or change?	
Evidence	
SMI B 31 Investigation of samples other than blood for parasites. Introduction (under protozoa).	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	**ACCEPT** *Giardia lamblia* changed to *Giardia intestinalis* based on NCBI taxonomy.

Comment number	3		
Date received	24/08/2017	**Professional body**	Society for Applied Microbiology
Section	a. 1.2 Specimen processing b. 4.5 Culture and investigation		
General comment			
a. The possibility of infection with verotoxin- or Shiga toxin-producing *E. coli* (such as O157) should be mentioned in this document, so far as to acknowledge that work on these organisms must be performed under Containment level 3 conditions (c.f. SMI ID 22). b. Chocolate agar may also be considered, in addition to blood agar. In addition to neomycin fastidious anaerobe agar, kanamycin blood agar (with added methadione and lysed blood) may also be considered. A metronidazole disc placed on a plate would speed confirmation of presence of anaerobes. Generally, it may be worthwhile considering the use of enrichment media (eg BHI or an anaerobic broth) in a situation where a very low number of microbes is expected. However, caution should be applied as the results of enrichment can be difficult to			

interpret.			
Recommended action	a. **NONE** Shiga – verotoxin producing *E. coli* are enteric (intraintestinal pathogens) and would not be found in a sterile bile sample. b. **NONE** This is not relevant for this document. If laboratories use other agar media not recommended in the UK SMI, they should ensure that they have validated and verified these. Blood agar will remain the recommended agar media in this document.		

Comment number	4		
Date received	25/08/2017	**Lab name**	Royal Cornwall Hospitals Trust
Section	4.5.1 and Appendix		
This UK SMI B 15 describes the processing and bacteriological investigation of bile. Considering that the processing of bile samples is very similar to the processing of other sterile fluids, the Standards Unit is considering merging the UK SMI B 15 with the UK SMI B 26 on the investigation of fluids from normally sterile sites. Would you agree with this merging? Please comment.			
Yes, merging the documents is a sensible idea. Typically sterile fluids are grouped and 'read' by one scientist. It will make the documentation simpler. Clearly the plates and incubation required would still need to be site specific.			
Recommended action	**NONE**		
General comment			
Slight confusion regarding when to read the anaerobic plates. Do you mean to say that the plates should be incubated for a minimum of five days, but it is possible to look at 48hrs?			
Evidence			
Please compare table in section 4.5.1 to the algorithm in Appendix.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** In the particular situations in which the prevalence of slow-growing microorganisms and anaerobes is higher; plates should		

	be read at 5 days and then left in the incubator/cabinet until day 14. Changed the wording to make it easier to understand.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 2			
Date received	16/08/2017	**Lab name**	Bacteriology - James Cook University Hospital
Date received	23/08/2017	**Lab name**	St. James's Hospital, Ireland

UK Standards for Microbiology Investigations

Investigation of tissues and biopsies from deep-seated sites and organs

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

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Bacteriology | B 17 | Issue no: 6.3 | Issue date: 05.01.18 | Page: 1 of 26

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UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Contents

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 7

SCOPE OF DOCUMENT 10

INTRODUCTION 10

TECHNICAL INFORMATION/LIMITATIONS 13

1 SAFETY CONSIDERATIONS 14

2 SPECIMEN COLLECTION 14

3 SPECIMEN TRANSPORT AND STORAGE 15

4 SPECIMEN PROCESSING/PROCEDURE 15

5 REPORTING PROCEDURE 20

6 NOTIFICATION TO PHE, OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 22

APPENDIX: INVESTIGATION OF TISSUES AND BIOPSIES FROM DEEP-SEATED SITES AND ORGANS 23

REFERENCES 24

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	13/05.01.18
Issue no. discarded.	6.2
Insert issue no.	6.3
Section(s) involved	**Amendment**
4.5.1 Culture media, conditions and organisms	Minor amendment to table.

Amendment no/date.	12/10.01.17
Issue no. discarded.	6.1
Insert issue no.	6.2
Section(s) involved	**Amendment**
4.5.1 Culture media, conditions and organisms.	Typing error corrected.

Amendment no/date.	11/13.12.16
Issue no. discarded.	6
Insert issue no.	6.1
Section(s) involved	**Amendment**
Specimen processing/procedure.	Section 4.4.2 Supplementary • Information has been updated on the preparation of tissue for examination in the case of suspected fungal infections along with a link to B 39 document for more information. Section 4.5.1 (culture media, conditions and organisms) media and incubation updated. • For Nocardiosis, the incubation temperature, atmosphere and time has

	been updated to reflect what is in the other UK SMI documents. • For *Legionella* species, the incubation atmosphere has been updated. • Footnotes have been added for clarity.
Appendix.	Updated to reflect section 4.5.1.

Amendment no/date.	10/08.04.16
Issue no. discarded.	5.3
Insert issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk. Title updated to include ‘from deep-seated sites and organs’. References reviewed throughout. Addition of lung tissue and biopsy for suspected infection with *Legionella* species.
Page 2.	Updated logos added.
Scope.	Scope updated to include rapid methods and links to relevant SMIs.
Introduction.	Reorganised for clarity. Specific tissue types placed into alphabetical order. Information regarding skin infection streamlined and information include in B11 – Investigation of swabs from skin and soft tissue infections.
Technical information/limitations.	Section on rapid methods included.
Safety considerations.	Safety considerations regarding Hazard Group 3 organisms amended. It is recommended that all Gram-negative coccobacilli from sterile sites should be processed in a Class I or Class II microbiological safety cabinet until Hazard Group 3 pathogens (ie Brucella) have been definitively excluded.
Specimen processing.	Samples for mycological examination must not be homogenised/ground.

Specimen processing/procedure.	Ideally, all grinding or homogenisation should be performed in a Class II exhaust protective cabinet. Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised. Addition of fluorescent staining technique. Section 4.5.1 (culture media, conditions and organisms) media and incubation updated. • Immunocompromised/suspected fungal infection changed to Sabouraud agar slope + chloramphenicol (35-37ºC 14d incubation, 28-30ºC 28d incubation). • Mycetoma addition of Sabouraud agar slope + chloramphenicol. • Nocardiosis blood agar 35-37ºC up to 7d. • Addition of *Legionella* species BMPA or alternative 35-37ºC up to 10d. • Mixed infection/local policy, addition of Mannitol Salt Agar. Section 4.6.1 (minimum level of identification) level of identification updated for β haemolytic streptococci, coagulase negative streptococci, enterobacteriaceae and pseudomonas. Consider sending staphylococci isolates from post mortem samples for toxin testing.
Reporting procedure.	Culture reporting statement updated.
Appendix.	Updated to reflect section 4.5.1.

UK SMI#: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

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https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Tissue, biopsy

This SMI describes the processing and investigation of tissues and biopsies from deep-seated sites and organs for bacteria and fungi.

In addition to culture methods, rapid methods including NAAT may be used.

For further information regarding investigation of infections caused by fungi, *Mycobacterium* species and parasites refer to:

B 39 - Investigation of dermatological specimens for superficial mycoses

B 40 - Investigation of specimens for *Mycobacterium* species

B 31 - Investigation of specimens other than blood for parasites

The following samples are not included in this document:

Tissue associated with orthopaedic implant infection (B 44 - Investigation of prosthetic joint infection samples).

Bone and soft tissue associated with osteomyelitis (B 42 - Investigation of bone and soft tissue associated with osteomyelitis).

Gastric biopsies (for the presence of *Helicobacter pylori)* (B 55 - Investigation of gastric biopsies for *Helicobacter pylori*).

This SMI should be used in conjunction with other SMIs.

Introduction

A biopsy may be defined as a portion of tissue removed from the body for further examination. With the increasing sophistication of clinical imaging and sampling devices there are few organs in the human body that cannot be biopsied. Tissue obtained at operation is particularly precious as the sampling procedure may not be repeatable. Ideally these specimens should be discussed with the laboratory prior to sampling to ensure that transport and processing are timely and appropriate tests are performed.

Biopsies and other tissue samples are obtained in 3 main ways:

- as a closed procedure usually through the skin (eg needle biopsy). Percutaneous biopsy samples are associated with particular problems; they are often very small, may miss the infected lesion and may be contaminated with skin flora
- as an open procedure at operation (eg during debridement of devitalised or infected tissue). Tissue obtained at operation is generally more rewarding to deal with, particularly when the purpose of surgery is to remove infected tissue
- at post mortem (eg tissue from the lungs of a patient with pneumonia). In many cases the primary purpose of sampling is to obtain tissue for histological examination. The microbiological yield from such samples is often low and they are commonly contaminated with enteric flora. Careful clinical interpretation of such isolates is required because they are often not significant

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Biopsies may be taken from chronically infected tissues and so, in addition to investigation for bacterial infection, they may also require investigation for fungi, *Mycobacterium* species and parasites.

Histological investigation will often inform the decision to investigate for particular classes of infection. For instance, the presence of caseating granulomata should raise the suspicion of tuberculous infection; similar appearances may be caused by deep fungal infection on occasion.

Tissues and biopsies are not easily repeatable specimens thus prolonged storage (1 month) of residual specimens may be critical in enabling the arrangement of any further appropriate investigations such as mycobacterial cultures or referral for 16S rDNA PCR.

Specific tissues[1]

Aortic aneurysm contents

Aortic aneurysm contents may be sent for the exclusion of an infective cause[2].

Artificial materials

Artificial materials may also be sent to the laboratory for investigation. Such materials include prosthetic cardiac valves, pacemakers, grafts, artificial joints and tissue implants.

Brain biopsies

Brain biopsies may sometimes be taken to differentiate non-infectious conditions from infection.

Corneas

Corneas should be examined in cases where deep seated eye infection is suspected. Refer to: SMI B 2 - Investigation of bacterial eye infections.

Donor heart valves or cornea rims

Donor heart valves or cornea rims need to be screened for bacterial infection prior to implantation.

Heart valves

Heart valves are submitted from patients with infective endocarditis undergoing valve replacement or at post mortem. Infected prosthetic valves may also be sent for culture. Where possible the results of these cultures should be correlated with blood cultures or serology.

In recent years PCR has been found useful in the diagnosis of infective endocarditis, detecting *Coxiella burnetii* in heart valve samples[3,4]. Duplex PCR has been successfully used to differentiate between *Coxiella burnetii* and other causes of infective endocarditis[5].

Lung biopsies (percutaneous, bronchoscopic, surgical or post mortem)[6]

Lung biopsies are classified by the method of entry or the reason for biopsy. They may be useful for infections caused by bacteria including *Actinomyces* species, *Nocardia*

species, *Legionella* species and *Mycobacterium* species and fungi, especially *Aspergillus* species, and *Pneumocystis jirovecii*. Pneumocystis pneumonia (PCP) occurs almost exclusively in patients who are immunocompromised. PCP may be diagnosed less invasively (usually with reduced sensitivity) by processing induced sputum or brochoalveolar lavage specimens. Refer to B 57 - Investigation of brochoalveolar lavage, sputum and associated specimens.

Lymph nodes

Excised lymph nodes are submitted for investigation of lymphadenitis, particularly suspected mycobacterial lymphadenitis. The most common cause in children under 15 years old is mycobacteria other than *Mycobacterium tuberculosis* (non-tuberculous Mycobacterium (NTM)) notably *Mycobacterium avium-intracellulare*. However, *Mycobacterium tuberculosis* may also be isolated from these and older patients[7]. Other important causes of lymphadenitis are toxoplasmosis; cat scratch disease which is caused by *Bartonella henselae*, a Gram negative organism endemic among domestic cats; and lymphogranuloma venereum - a sexually transmitted chlamydial infection[8]. All of these conditions are perhaps best diagnosed by a combination of histological and serological investigations, coupled with molecular diagnostic testing where available (eg NAAT for Toxoplasma genome, offered by the Toxoplasma Reference Laboratory https://www.gov.uk/government/collections/toxoplasma-reference-laboratory-trl).

Placental specimens and products of conception

Products of conception and placental specimens are submitted for the investigation of septic abortion and listeriosis. *Listeria monocytogenes* may cause serious infection in pregnant women, neonatal infants and patients who are immunocompromised[9,10]. In pregnant women septicaemia caused by *L. monocytogenes* presents as an acute febrile illness that may affect the fetus[10]. This may lead to systemic infection (granulomatosis infantisepticum), stillbirth and neonatal meningitis. Products of conception, placenta and neonatal screening swabs should be examined for this organism. Routine culture of vaginal swabs for *L. monocytogenes* is not usually performed although it may be useful in suspected cases. Blood cultures are indicated. Serological investigations have no place in the diagnosis of listeriosis (see B 28 - Investigation of genital tract and associated specimens)[9].

Septic abortion may result in serious maternal morbidity and may be fatal[10]. Uterine perforation, presence of necrotic debris, and retained placental products can lead to infection. Most infections are polymicrobial and involve anaerobes. Clostridial sepsis complicating abortion is potentially lethal. *Clostridium* species are part of the normal vaginal flora in some women.

Skin biopsies

Skin biopsies may be submitted for the investigation of bacterial and fungal skin and soft tissue infection, and tissue parasites such as *Onchocerca volvulus*, *Mansonella streptocerca* and *Leishmania* species (B 31 - Investigation of specimens other than blood for parasites). They are also used to confirm cases of swimming pool or fish tank granuloma, a chronic skin infection which results from infection with *Mycobacterium marinum*, and is associated with injury and contact with water for swimmers and keepers of tropical fish[11] (B 40 - Investigation of specimens for *Mycobacterium* species).

Necrotising fasciitis is limited by the deep fascia. The infection spreads widely and rapidly due to the absence of internal barriers in the fascia. The infection can be fatal in a very short time. Some cases occur post-operatively or in patients with underlying clinical conditions such as malignancy. Some authorities consider that it exists as two types. Type I is due to infection by a polymicrobial mixture with aerobic and anaerobic organisms (group A streptococci, anaerobes, *S. aureus* and members of the Enterobacteriaceae). Type II (haemolytic streptococcal gangrene) is due to infection with group A streptococci[12].

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[13,14]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Rapid methods

To improve sensitivity and reduce turnaround times, rapid identification and sensitivity tests may be performed in conjunction with routine methods where appropriate. A variety of rapid identification and sensitivity methods have been evaluated; these include molecular techniques and matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF)[15-17]. It is important to ensure that fresh cultures of pure single isolates are tested to avoid reporting misleading results.

Laboratories should follow manufacturers’ instructions and all rapid tests must be validated and be shown to be fit for purpose prior to use.

1 Safety considerations[13,14,18-32]

1.1 Specimen collection, transport and storage[13,14,18-21]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[13,14,18-32]

Containment Level 2.

Where infection with a Hazard Group 3 organism eg *Mycobacterium tuberculosis*, *Brucella abortus*, *Histoplasma capsulatum, Coccidioides* species, *Blastomyces dermatitidis, Paracoccidioides brasiliensis, Talaromyces* (previously *Penicillium*) *marneffei, Cladophialophora* species*, Fonsecea* species and *Rhinocladiella mackenziei* is suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

It is recommended that all Gram-negative coccobacilli from sterile sites should be processed in a Class I or Class II microbiological safety cabinet until Hazard Group 3 pathogens (ie *Brucella*) have been definitively excluded[33].

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[24].

Grinding and homogenisation of all specimens must be undertaken in a microbiological safety cabinet. Wherever possible, the use of sterile scissors is recommended in preference to a scalpel blade.

Note: Samples for mycological examination must not be homogenised/ground.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Tissue, biopsy

2.2 Optimal time and method of collection[1]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[1].

A medical practitioner will normally collect the specimen.

Collect specimens into appropriate CE marked leak proof containers and place in sealed plastic bags.

General

If specimen is small, place it in sterile water to prevent desiccation.

Note: Specimens received in formol-saline are not suitable for culture.

Note: Ensure that the retention and disposal of tissues complies with the Human Tissue Act 2004.

Suspected *Legionella* species (lung tissue and biopsy)

If specimen is small place it in sterile water to prevent desiccation.

Note: This would not be appropriate for specimens undergoing processing for diagnosis by molecular methods.

Note: Avoid the use of saline, as it is known to be inhibitory to *Legionella* species.

2.3 Adequate quantity and appropriate number of specimens[1]

The specimen should, ideally, be large enough to carry out all microscopy preparations and cultures.

Minimum specimen size will depend on the number of investigations requested.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport and storage[13,14]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[1].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[1].

The volume of the specimen influences the transport time that is acceptable. Larger pieces of tissue maintain the viability of anaerobes for longer[34].

Tissues and biopsies are not easily repeatable specimens thus prolonged storage (1 month) of residual specimens may be critical in enabling the arrangement of any further appropriate investigations such as mycobacterial cultures or referral for 16S rDNA PCR.

4 Specimen processing/procedure[13,14]

4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for fungi (B 39 - Investigation of dermatological specimens for superficial mycoses) and *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species), and examination for parasites (B 31 - Investigation of specimens other than blood for parasites) depending on clinical details.

If there is insufficient specimen for all investigations, they should be prioritised according to clinical indications after consultation with a medical microbiologist.

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

Standard

Grind or homogenise specimen with, as appropriate, using a sterile tissue grinder (Ballotini beads), a sterile scalpel or (preferably) sterile scissors and petri dish. The addition of a small volume (approximately 0.5mL) of sterile, filtered water, saline, peptone or broth will aid the homogenisation process.

Ideally, all grinding or homogenisation should be performed in a Class II exhaust protective cabinet.

Note: Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised[35].

4.3.2 Supplementary

N/A

4.4 Microscopy

4.4.1 Standard

N/A

4.4.2 Supplementary

Gram stain

Homogenised specimens

(See section 4.3.1 for method of homogenisation).

Place one drop of specimen on to a clean microscope slide with a sterile pipette.

Spread this with a sterile loop to make a thin smear for Gram staining.

Non-homogenised specimens

Prepare a touch preparation - use sterile forceps to grasp pieces of specimen, touch the sides of one or more pieces of the specimen to a clean microscope slide for Gram staining. Group the touch preparations together for easier examination. This sample should not be used for culture.

See TP 39 - Staining procedures.

Fluorescent staining technique

Follow kit manufacturers' instructions.

Legionella

For suspected *Legionella* species (lung tissue and biopsies) homogenise specimens as in section 4.3.1.

Using a sterile pipette, place one drop of homogenised specimen onto a clean PTFE microscope slide.

Spread the drop with a sterile loop to make a thin smear for fluorescent staining.

Suspected fungal infections

For suspected fungal infections finely cut specimens as in section 4.3.1.

Place a small portion of tissue in a sterile Eppendorf tube and add equal proportions of 10-30% KOH and Calcofluor white (0.1%) solution. Leave to digest for at least 20 min or less at room temperature. After digestion, the tissue should be squashed to produce a single layer of cells.

Using a sterile pipette, place the digested tissue on a glass slide, and examine under a fluorescent microscope. Note the type of structures seen to correlate with subsequent culture results ie pseudohyphae, true hyphae, yeast forms, other fungal elements.

For more information, refer to TP 39 - Staining procedures and B 39 - Investigation of dermatological specimens for superficial mycoses.

4.5 Culture and investigation

Homogenised specimens

Inoculate each agar plate and enrichment broth with homogenised or ground specimen (see Q 5 – Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Non-homogenised specimens

Inoculate each agar plate with the cut pieces of tissue (see Q 5 – Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp. °C	Atmos.	Time		
All clinical conditions	Tissue Biopsy	Blood agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
		CLED/ MacConkey agar	35-37	Air	18-24hr	≥18hr	
		Selective anaerobic agar	35-37	Anaerobic	5d	≥40hr and at 5d	Anaerobes
		Fastidious anaerobic, cooked meat broth or equivalent.	35-37	Air	Up to 5d	N/A	Any organism
		Subculture if evidence of growth (≥40hr), or at day 5	35-37	As above	As above	As above	

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp. °C	Atmos.	Time		
		to above media (excluding MacConkey agar)					
	For these situations, add the following:						
If microscopy suggestive of mixed infection	Tissue Biopsy	Selective anaerobic agar with metronidazole disc 5µg	35-37	Anaerobic	5d	≥40hr and at 5d	Anaerobes
Actinomycosis	Tissue Biopsy	Blood agar supplemented with metronidazole and nalidixic acid	35-37	Anaerobic	10d	≥40hr, at 7d and 10d	*Actinomyces* species
Immunocompromised, or suspected fungal infection	Tissue Biopsy	Sabouraud agar slope + chloramphenicol	35-37 and 28-30	Air	14d 28d	daily#	Yeasts Moulds
Mycetoma	Tissue Biopsy	Lowenstein-Jensen slope / Blood agar or Sabouraud agar slope + chloramphenicol	35-37 28-30	Air Air	up to 28d	Every 3-4 days	Aerobic *Actinomycetes* species Yeasts Moulds
Nocardiosis	Tissue Biopsy (bronchoal veolar lavage)	Blood agar	35-37	5-10% CO_2	16-48hr	daily	*Nocardia* species**
Suspected Legionellosis	Tissue Biopsy	BMPA or BCYEA or alternative Legionella agar	35-37	Moist Atmos*	Up to 10d	3d, 7d and 10d	*Legionella species*
	Optional media:						
When clinical details or when microscopy suggestive of mixed infection or dependent on local policy	Tissue Biopsy	Staphylococci/ Streptococci selective agar or Mannitol Salt Agar (not for Streptococcus)	35-37	Air	40-48hr	daily	*S. aureus* Streptococci
Other organisms for consideration – Fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), *H. pylori* (B 55 - Investigation of gastric biopsies for *Helicobacter pylori*), *Listeria* species, *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).							

#Agents of exotic imported mycoses eg *Histoplasma capsulatum* and some *Cryptococcus* isolates may take up to 8 weeks to grow; adequate humidification of incubators will be necessary[36,37].

*It should be noted that incubation in 2-5% CO_2 can enhance growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*[38]. This low level of CO_2 will not affect the growth of *L. pneumophila,* but CO2 levels higher than 5% may inhibit growth.

** If laboratories choose to use *Legionella* selective agar plates such as BCYE agar as supplementary media for isolation of *Nocardia* species, its inclusion should be subject to the results of local validation. The document, ID10: Identification of aerobic actinomycetes recommends that if selective agar plates are used, they should be incubated for 2 to 3 weeks.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Actinomycetes	genus level ID 10 – Identification of aerobic *Actinomycetes* species ID 15 – Identification of anaerobic *Actinomycetes* species
Anaerobes	"anaerobes" level ID 8 - Identification of *Clostridium* species
β-haemolytic streptococci	species level
Coagulase negative staphylococci	"coagulase negative" level
Enterobacteriaceae	species level
Pseudomonads	species level
S. aureus	species level (consider Panton-Valentine leukocidin (PVL) and toxin testing if appropriate clinical details) (consider toxin testing on samples from post mortems)
S. anginosus group	*S. anginosus* group level
Yeast	species level
Mould	species level
Legionella species	species level
Mycobacterium species	species level B 40 - Investigation of specimens for *Mycobacterium* species
Parasites	species level B 31 - Investigation of specimens other than blood for parasites

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

CLSI breakpoints are available for *Candida* species and moulds.

4.8 Referral for outbreak investigations

Refer to British Society for Antimicrobial Chemotherapy (BSAC) guidelines.

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Consider sending *S. aureus* isolates for toxin testing where appropriate clinical details are provided. For example, isolates from post mortems where the specimen is suspected to be the cause of death should be sent for toxin testing.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Gram stain

Report on WBCs and organisms detected.

Legionella immunofluorescence

Legionella pneumophila detected by immunofluorescence **or**

Legionella pneumophila not detected by immunofluorescence

Fungal fluorescent stain

Report on type of fungal element seen.

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

The following results should be reported:

- clinically significant organisms isolated
- other growth with appropriate comment, eg No significant growth
- absence of growth

Also, report results of supplementary investigations.

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Legionella

Final written or computer generated reports should follow preliminary/verbal reports within 3 - 10 days stating, if appropriate, that a further report will be issued.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[39,40], or equivalent in the devolved administrations[41-44]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

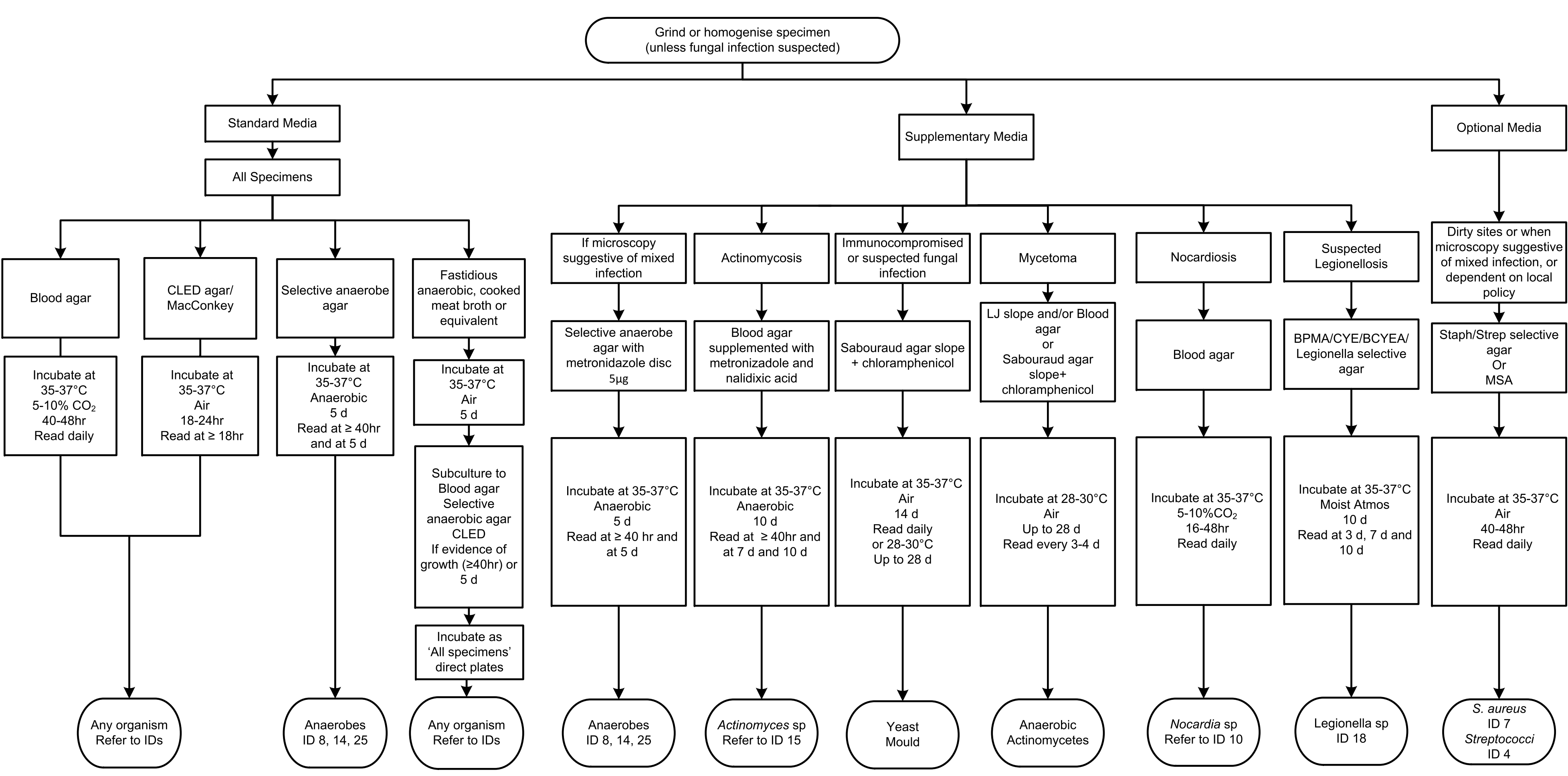
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[41,42], Wales[43] and Northern Ireland[44].

Appendix: Investigation of tissues and biopsies from deep-seated sites and organs



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UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

B 17 Investigation of tissues and biopsies from deep-seated sites and organs

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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A50711295

1st Consultation: 23/08/2013 – 15/11/2013

Version of document consulted on: B 17dg+

Proposal for changes

<table>
<tr><td>Comment number</td><td>1</td><td colspan="2"></td></tr>
<tr><td>Date received</td><td>29/10/2013</td><td>Lab name</td><td>Kings College Hospital-representing MSTAG</td></tr>
<tr><td>Section</td><td colspan="3">Various</td></tr>
<tr><td colspan="4">Comment</td></tr>
<tr><td colspan="4">General comments:
a. Lots of cross references, almost unreadable perhaps Tissue and Bone UK SMIs could be amalgamated.
b. 1.2.1 Delays over 24hr, the group would like clarification of the reference quoted as it seem to be from a text book.
c. 2.5.2 The note regarding fungal cultures may be better placed in section 2.5.1.
d. 2.5.3 FAB sub-cultured at >40hr, but incubation time quoted is 5 days.
e. Sabouraud agar 40-48hr this is inconsistent with the flow chart, should it be 5 days as a minimum?
f. FAA+ neomycin is in ‘all samples’ and ‘if microscopy suggestive of mixed infection’.
g. Flowchart - some typos seen.
h. Grind/homogenise - not if Fungi are sought.
i. BSAC guidelines quoted-should this be more general as EUCAST used widely.</td></tr>
<tr><td colspan="4">Health benefits</td></tr>
<tr><td colspan="4">No.</td></tr>
<tr><td>Recommended action</td><td colspan="3">a. PARTIAL ACCEPT
Most of the cross reference have been moved to the scope and are not repeated throughout the document. The document title and scope of the document has been updated.
b. ACCEPT
This has been removed as no evidence can be found to support this statement. Alternative recommendations are being sought.
c. ACCEPT
The text has been moved to section 4.4.1.
d. ACCEPT
Table and flowchart updated in final version. Broths are</td></tr>
</table>

	subcultured if evidence of growth (≥40hr), or at day 5. e. **PARTIAL ACCEPT** Following discussion incubation has been updated (in final version) to 14 days at 35-37ºC or 28 days at 28-30ºC. f. **NONE** If microscopy suggestive of mixed infection a metronidazole disc is added g. **ACCEPT** Flowchart reviewed and updated h. **ACCEPT** Text updated in section 4.4.1. i. **ACCEPT** This has been updated in the document.

Comment number	2		
Date received	30/10/2013	**Lab name**	Manchester PHL
Section	Scope and specific tissues		
Comment			
a. The scope is unclear and limited; although it states the document is on bacteriological processing there is quite a lot of material on parasites and fungi. b. There does not seem to be anything on viruses even in the background, although corneas, intestinal biopsies, lung biopsies, brain biopsies often have viral PCRs done. c. PCR is not mentioned in the document even though many of the bacteria and fungi mentioned will be best diagnosed by PCR; heart valves and liver granulomata for instance would often have *C. burnetii* PCR done and *P. jirovecii* will be tested by PCR in many cases. Many biopsies are tested for bacterial and fungal pathogens by 16S and 18S PCR. d. Brain biopsy has been overlooked in the sample types (it is only mentioned in safety section with regard to exotic pathogens), but it is still taken for undiagnosed mass lesions so is relevant to this document in looking at the differentiation between bacterial, fungal and toxoplasma abscesses versus lymphoma etc.			
Financial barriers			
No.			
Recommended action	a. **ACCEPT** Reference to fungi and parasites removed from the majority of the document. Scope updated. b. **PARTIAL ACCEPT**		

	It is specified in the scope that this SMI covers bacteria and fungi only. Reference to NAATs has been included in the document. c. **ACCEPT** Reference to NAATs has been included throughout. d. **ACCEPT** Brain biopsies added to specific tissue section.

Comment number	3		
Date received	06/11/2013	**Lab name**	Royal Alexandra Microbiology Department
Section	2.5.3		
Comment			
a. I think it is unnecessary to put up a MacConkey or CLED plate routinely with all such samples, many of which will be sterile. b. Most organisms will grow on the blood and can be separated from there. A chocolate plate for any fastidious organisms would be more useful. c. CLED as an addition for certain clinical details may be useful.			
Evidence			
Experience of culturing samples.			
Recommended action	a-c. **NONE** Following discussions, the group agreed that the following combination of plates was most appropriate for all clinical conditions: blood agar, CLED/MacConkey/Selective anaerobic agar and a fastidious anaerobe broth.		

2nd Consultation: 06/01/2015 – 26/01/2015

Version of document consulted on: B 17do+

Proposal for changes

Comment number	1		
Date received	15/01/2015	**Lab name**	Nottingham University Hospitals
Section	1.2 and 4.6.1		
Comment			
a. 1.2- Suggest change wording as per B 14 -totally impractical to state any sample			

	from brain abscess or any site from a patient with a travel history to America, Africa Asia etc is processed in full containment level 3, because of a potential but extremely rare infection of Rhinocladiella infection. Keep statement where infection with hazard group 3 organisms in 4.6.1. b. Suggest from such deep-seated sites both Pseudomonads and Coliforms should be identified further, Pseudomonads to species levels and Coliforms to at least genus level.
Recommended action	a. **ACCEPT** The safety section will be reviewed and the text regarding brain abscesses and *Rhinocladiella mackenziei* removed. b. **ACCEPT** It was agreed that Pseudomonads and Enterobacteriaceae should be identified to species level. The table in 4.6.1 will be updated accordingly.

Comment number	2		
Date received	20/01/2015	**Lab name**	Public Health laboratory Manchester
Section	Specific tissues		
Comment			
a. Lung biopsies...: PCP may be diagnosed less invasively, but usually with reduced sensitivity, by processing induced sputum or bronchoalveolar lavage specimens. PCP is usually diagnosed by PCR nowadays with high sensitivity and specificity on samples such as sputum (induced or otherwise) and BAL. Suggest rewrite along the lines of: PCP is usually diagnosed less invasively using sensitive PCR methods on samples including induced sputum or bronchoalveolar lavage. b. Aspergillus culture has poor sensitivity - suggest highlighting use of PCR and galactomannan in diagnosis on BAL samples. c. Throughout this section the value of PCR might be highlighted where appropriate eg Heart valves - Q fever PCR, 16S PCR, Lymph nodes - Toxoplasma PCR is available in a number of centres, not just the Swansea reference laboratory, etc.			
Evidence			
One example for PCP: J Clin Microbiol. 2011 May;49(5):1872-8. doi: 10.1128/JCM.02390-10. Epub 2011 Mar2.Multicenter, prospective clinical evaluation of respiratory samples from subjects at risk for Pneumocystis jirovecii infection by use of a commercial real-time PCR assay. M.Hauser PM(1), Bille J, Lass-FlÃl C, Geltner C, Feldmesser M, Levi M, Patel H,Muggia V, Alexander B, Hughes M, Follett SA, Cui X, Leung F, Morgan G, Moody A,Perlin DS, Denning DW. One example for aspergillus: J Clin Microbiol. 2012 Nov;50(11):3652-8. doi: 10.1128/JCM.00942-12. Epub 2012Sep 5.Diagnostic accuracy of PCR alone compared to galactomannan in bronchoalveolarlavage fluid for diagnosis of invasive pulmonary aspergillosis: a systematic review. Avni T(1), Levy I, Sprecher H, Yahav D, Leibovici L,			

Paul	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **PARTIAL ACCEPT** It was felt that this was a valid point. However, the information is better placed in (and is included in) B 57: Investigation of brochoalveolar lavage, sputum and associated specimens. A cross reference to B 57 will be included in the text. b. **PARTIAL ACCEPT** It was agreed that this was outside of the scope of the document. This is information is included in B 57. A cross-reference to B 57 will be included in the text. c. **ACCEPT** Text regarding PCR will be included where appropriate.

Comment number	3		
Date received	23/01/2015	**Professional body**	UKCMN
Section	a. Section 1.2 b. Section 4.4.2 c. Section 4.5.1 d. Section 5.1 e. Section 4.4.2 f. Appendix		
Comment			
a. Section 1.2 Replace Penicillium marneffei with Talaromyces (previously Penicillium) marneffei, Add 'and Cladophialophora species' so it reads: (to cover Rhinocladiella mackenziei 27 and Cladophialophora species), Add sentence after safety cabinet so it reads: Grinding and homogenisation of all specimens must be undertaken in a microbiological safety cabinet. NB Samples for mycological examination must not be homogenised. b. Section 4.4.2 Add a paragraph on fungal Fluorescent staining technique: Fluorescent staining for fungi. For suspected fungal infection place a small portion of tissue in a sterile Eppendorf tube with a few drops of 20% KOH, place in a heat block for 20 min to soften. Using a sterile pipette place the softened tissue on a glass slide, add a drop of calcoflour/blankophor and view under a fluorescent microscope. Note the type of structures seen to correlate with subsequent culture results ie pseudohyphae,			

true hyphae, yeast forms, other fungal elements. c. Section 4.5.1 At the bottom of the table add 'and some Cryptococcus isolates ' so it reads: eg Histoplasma capsulatum, and some Cryptococcus isolates. d. Section 5.1 Add a reporting line for fungi seen: Mycology “report on type of fungal elements seen Appendix Replace top box of the flow diagram with Grind or homogenise a specimen unless a fungal infection is suspected. e. Section 4.5.1 table “Immunocomp host sab agar listed, but shouldn't this be a sab slope + chloramphenicol- Similarly for mycetoma there should be a sab+chloramphenicol slope and this should be incubated at 30°C. f. Appendix - The mycetoma decision tree needs to include mould media, the immunocomp tree should have chloramph in the media and the temperature should be 30C as well and incubate for up to 28 days at least.
Financial barriers
No.
Health benefits
No.

Recommended action	a-f. **ACCEPT** The document will be updated to reflect the comments made.

Comment number	4		
Date received	23/01/2015	**Lab name**	Truro
Section	a. Page 11 b. Page 19		
Comment			
a. Pg 11, 1.2 - Penicillium marneffei is now named Talaromyces marneffe. b. Pg 19 - Sabouraud agar also incubated at 28-30°C.			
Recommended action	a. **ACCEPT** Text updated. b. **ACCEPT** Section 4.5.1 and flowchart updated.		

Comment number	5		
Date received	26/01/2015	**Professional body**	IBMS
Section	a. Section 1.2 b. Appendix 1		

	c. Section 4.7
Comment	
a. Section 1.2 Specimen processing Paragraph starts, ‘It is recommended that all Gram-negative coccobacilli from (TEXT MISSING HERE) should be processed….’ Additional text needs to be added to say what the specimen is from. b. Appendix 1 Typo in chart under selective media – should say Nocardiosis, not Norcardiosis, and the bubble at the bottom that says ‘7d Norcardia sp…,’ also needs correcting. c. Under the antimicrobial susceptibility testing each document make reference to BSAC or EUCAST which is fine for bacterial pathogens. However, for Candida and Moulds (which are mentioned in the text) only CLSI breakpoints apply.	
Recommended action	a. **ACCEPT** Text corrected. b. **ACCEPT** Text corrected. c. **ACCEPT** Text added to section 4.7.

Comments received outside of consultations

Comment number	1		
Date received	22/05/2013	**Lab name**	Belfast
Section	Page 22		
Comment			
We have been revising SOPs and referred to SMI Bacteriology B 17 (Investigation of Tissues and Biopsies) for guidance. While studying flowchart on page 22 of the document it was noted that stream for ‘all samples’ does not have a recommendation for enrichment (?fastidious anaerobe broth) but rather two adjacent cells refer to prolonged incubation on solid media. Apologies if you have already been inundated with notifications of this observation.			
Recommended action	**ACCEPT** This has been addressed. The flowchart now includes FAA broth for all samples.		

Respondents indicating they were happy with the contents of the document

Overall number of comments: 4			
Date received	02/09/2013	**Lab name**	R&D, Department of Microbiology,

			Leeds General Infirmary
Date received	07/11/2013	**Lab name**	Royal Oldham Hospital
Date received	06/01/2015	**Lab name**	Microbiology Queen Elizabeth Hospital LGHT Woolwich SE18 4QH
Date received	21/01/2015	**Lab name**	Northern Health and Social Care Trust

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UK Standards for Microbiology Investigations

Investigation of intravascular cannulae and associated specimens

NICE accredited
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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 20 | Issue no: 6.1 | Issue date: 27.06.17 | Page: 1 of 24

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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Contents

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 11

1 SAFETY CONSIDERATIONS 12

2 SPECIMEN COLLECTION 12

3 SPECIMEN TRANSPORT AND STORAGE 13

4 SPECIMEN PROCESSING/PROCEDURE 13

5 REPORTING PROCEDURE 18

6 NOTIFICATION TO PHE, OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 19

APPENDIX: INVESTIGATION OF INTRAVASCULAR CANNULAE AND ASSOCIATED SPECIMENS 20

REFERENCES 21

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	10/27.06.17
Issue no. discarded.	6
Insert issue no.	6.1
Section(s) involved	**Amendment**
Introduction.	Minor textual additions.
Section 2.2.1	Edited for clarity.

Amendment no/date.	9/30.11.15
Issue no. discarded.	5.2
Insert issue no.	6
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. Hyperlinks updated to gov.uk. Reorganisation of some text. Edited for clarity. Minor textual changes. All hyperlinked documents updated with the correct address.
Page 2.	Updated logos added.
Scope of document.	This document has been updated to exclude the investigation of specimens such as pacing wires and ventricular assist devices.
References.	References updated.
Appendix.	The flowchart has been updated for easy guidance.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (2017). Investigation of intravascular cannulae and associated specimens. UK Standards for Microbiology Investigations. B 20 Issue 6.1. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Line tips eg CVP or Hickman lines, swabs of cannula insertion sites

Note: Peripheral lines are not suitable specimens for cultures and should not normally be sent to the laboratory for testing.

This SMI describes the processing and bacteriological investigation of intravascular cannulae and associated specimens. This document does not include the investigation of specimens such as pacing wires and ventricular assist devices. It should also be noted that the words “cannulae” and “catheters” are widely used interchangeably and in this document, the same applies to the two terms.

Occasionally blood may be used and for more information, refer to B 37 - Investigation of blood cultures (for organisms other than *Mycobacterium* species).

This SMI should be used in conjunction with other SMIs.

Introduction

The use of indwelling cannulae for reliable intravascular access is an essential feature of modern health care for both monitoring and intervention. Insertion of intravascular cannulae allows continuous and painless access to the circulation for administration of fluids and electrolytes, medications, blood products and nutritional support. In addition the intravascular access can be used for blood sampling, haemodynamic monitoring, haemodialysis and haemofiltration. Each year, millions of intravascular devices are used in acutely or chronically ill hospitalised patients around the world. These devices come in various lengths to suit peripheral or central insertion and can have single or multiple lumens. Although the vast majority of these devices are cannulae for peripheral use, central venous or arterial catheters are also used especially in patients with difficult peripheral access or when haemodynamic monitoring is indicated.

Cannula-related infections are amongst the most important nosocomial infections. Skin colonisation (which is often asymptomatic) acts as a precursor of systemic or localised infection. The overall incidence of infection related to the use of intravascular cannulas is about 1%, however this figure may be as high as 4-8% for central venous cannulas used for total parenteral nutrition. In high risk patients, central venous line infections carry a significant mortality rate and a high cost[1].

Intravascular device related blood stream infection is a significant clinical problem. Evidence Based Practice for Infection Control (EPIC) guidelines have been issued by the Department of Health for the prevention of hospital acquired infections associated with the use of central venous catheters[2]. Catheter-related blood stream infections (CR-BSI) can be defined as the isolation of the same organism ie identical species from the colonised catheter and peripheral blood in a patient with accompanying clinical signs and symptoms of bloodstream infection (BSI) and no other apparent source of BSI[3,4]. The guidelines recommend several practices and strategies for reducing the risk of CR-BSI, including catheter type, site of insertion, optimum aseptic technique, good catheter care, and the appropriate use of antimicrobial coated or impregnated central venous catheters (CVCs).

Types of cannulae

Specific examples of descriptions of cannulae (or catheters), defining their siting, use or design, include:

- **peripheral lines** – these lines are usually inserted into the veins of the forearm or the hand to administer medication, fluids or nutrition eg Venflons, Abbocaths and Parenteral nutrition catheters. They can be used either short term or long term
- **midline catheters** – these are inserted via the antecubital fossa into the proximal basilic or cephalic veins. They do not enter the central veins and are for short-term use to sample blood or administer fluids intravenously
- **central lines** – these are inserted into central veins (such as triple lumen, subclavian lines, jugular lines or less commonly femoral lines) with the tip residing in the vena cava. This permits intermittent or continuous infusion of irritant, vesicant or hyper-osmolar drugs/fluids and/or access into the venous system and can be used short term or long term eg peripherally inserted central catheter (PICC).There are various subtypes of central lines and their uses. They are as follows:
    - monitoring lines, eg central venous pressure lines, Swan Ganz lines, arterial lines
    - long term access such as chemotherapy, antibiotics, blood sampling, continuous renal replacement therapy (CRRT), haemodialysis eg Hickman lines, Broviac lines, Groshong lines, Hickman-like catheters such as Lifecath, RedoTPN, etc
    - miscellaneous eg dialysis lines (Vascath used for haemofiltration), and umbilical cannulae for exchange transfusions in neonates
    - implantable ports eg TIVAD, Portacath. These are subcutaneous ports or reservoirs with self-sealing septum which are tunnelled beneath the skin and accessed by a needle through intact skin. They are implanted in subclavian or internal jugular veins. It is associated with low rates of infection
    - antimicrobial coated or impregnated CVCs: recent studies have demonstrated that antimicrobial coated or impregnated CVC can reduce the incidence of catheter colonisation and CR-BSI in appropriate situations[2]
- **catheter hubs, stopcocks and needle-free connectors** are used to reduce the risk of accidental needle puncture or biological contamination in clinical settings. Manufacturer's recommendations should be adhered when using these devices[5]

Cannula-related bacteraemia or cannula-related fungaemia

The cannula may be the source of a bacteraemia or fungaemia. This is likely to be so if it is infected with the same organism as that isolated from a blood culture, usually in the absence of an identifiable alternative focus of infection, and when cultures from infusions are negative[6]. Infection of intravenous cannulae may lead to widespread dissemination of infection. More usually the patient develops a fever and may become generally unwell.

Cannula-related sepsis

This is defined as the presence of clinical sepsis when two or more of the following occur; such as fever, leucocytosis, or hypotension, documentation of a catheter isolate (irrespective of quantitative count), and negative blood cultures obtained within 48hr before and 24hr after catheter removal. There is usually no other source of sepsis demonstrated, and this resolves following catheter removal[7].

Localised infection

This can occur at the insertion site and subcutaneous track of the device[8]. Clinical signs of infection include erythema, exudate formation, oedema and thrombophlebitis. The patient may complain of pain or irritation at the insertion site, and may become pyrexial. This infection is caused by pyogenic bacteria such as *Staphylococcus aureus*.

Cannula removal and culture

According to the Centres for Disease Control and Prevention - Infectious Diseases Society of America (CDC-IDSA) guidelines, it recommends that culture of tips should only be done when CRBSIs are suspected. Cannula tip culture gives valuable information but necessitates the removal of the cannula. This can sometimes result in the loss of venous access that can interfere seriously with the medical management of the patient, although sometimes catheter removal is necessary to gain control of a catheter-related infection, especially with certain organisms, such as *Candida* species[9,10].

Cannula associated swabs (eg swabs of catheter insertion sites) may be employed as alternative specimens. However, routine investigation of cannula associated swabs from asymptomatic patients is of dubious value.

When skin and blood culture results concur, removal of the cannula is usually recommended (depending on clinical settings and organism identified), although this may not happen in practice unless clinical sepsis unresponsive to antibiotics is present. Quantitative and semi-quantitative culture methods have been described for these sites, but are not recommended in this SMI[11-14].

An alternative method of investigating cannula-associated infection that preserves central venous access is to take samples of blood simultaneously through the cannula and from a peripheral vein[15]. For added specificity, both samples are cultured quantitatively. If the concentration of organisms in the blood from the central line is equal to or greater than 10 times the concentration of organisms in blood from the peripheral vein, then central venous cannula infection is diagnosed[16]. The latter methodology has not been widely adopted because of its complexity, cost and limited added value.

Infections and organisms

The incidence of infection is related to the length of time the cannula remains *in situ*. The catheter tip may be infected secondarily by organisms already infecting the hub or insertion site which track down the catheter lumen or tunnel; but it may also acquire organisms from fluids passing through it or from the bloodstream itself. Colonisation of cannulae is a far commoner source of CR-BSI than contaminated infusate. Organisms causing cannula-related infections may be acquired from[17,18]:

- patients' microflora

- hands of staff
- contaminated disinfectants
- contaminated hub
- bacteraemia due to other causes
- contaminated intravenous fluids
- ward air

Most central venous line-associated infections are caused by organisms from the skin near the exit site which gains access to the intravascular segment of the cannula.

Organisms isolated from cannula tips and swabs commonly associated with cannula sites in descending order of frequency include[19,20]:

- coagulase negative staphylococci (CoNS)
- *Staphylococcus aureus* including MRSA
- enterobacteriaceae
- enterococci
- pseudomonads
- *Corynebacterium* species
- streptococci
- *Bacillus* species

Fungi may be isolated including:

- *Candida albicans* and other yeasts
- *Aspergillus* species
- *Fusarium* species
- *Malassezia furfur* (in patients receiving intralipid infusions)

Coagulase negative staphylococci

Coagulase negative staphylococci (CoNS) are the most frequent causes of cannula-related infections. It should also be noted that intraluminal colonisation of the common skin flora from repeated puncture through the septum may account for the high percentage of CoNS[21]. They can produce extracellular slime that facilitates adherence and may limit the access of antibiotics, and may reduce the host's inflammatory response. If a patient has a central venous cannula and coagulase negative staphylococci are isolated from multiple sets of blood cultures, infection with the organism must be considered seriously. However, there may be difficulty in interpretation of the significance of these isolates, as coagulase negative staphylococci are commonly isolated from contaminated blood cultures.

Any organism isolated in significant numbers should be considered as of potential significance when using methods of quantitative culture[1,22].

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[23,24]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS)

Direct identification of bacteria and yeast from blood culture bottle broth by MALDI-TOF methods is promising and it has the potential to speed the identification process in the laboratory thereby reducing turnaround times as well as resulting in significant improvements to patient care. Rapid identification of blood culture contaminants may also allow more rapid discontinuation of unnecessary antimicrobial therapy. Different protocol types have been reported by several studies to accurately identify the microorganisms present in positive blood culture broth; however, a lack of standardised protocols and the use of different software for mass analysis and different blood culture bottles make it difficult to compare the performances of the different methods[25-28].

1 Safety considerations[23,24,29-43]

1.1 Specimen collection, transport and storage[23,24,29-32]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags[44].

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[23,24,29-43]

Containment Level 2 organisms.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[35].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Line tips eg CVP or Hickman lines, swabs of cannula insertion sites

2.2 Optimal time and method of collection[45]

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[45].

Cannulae should be collected in appropriate CE marked leak proof containers and transported and processed as soon as possible.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[44,46-49].

2.2.1 Correct specimen type and method of collection

Cannulae

Disinfect the skin around the cannula entry site, remove cannula using aseptic technique, and ideally cut off 4cm of the tip into an appropriate CE marked leak proof container using sterile scissors[8]. Place in sealed plastic bags for transport.

Note 1: skin disinfection procedures depend on local protocols and may vary.

Note 2: cannulae should only be sent if there is evidence of infection.

Swabs

Sample the inflamed area / exudate around the catheter insertion site using an appropriate swab.

Blood

At least two blood cultures should be obtained when catheter infection is suspected by peripheral venepuncture[20]. For more information on blood cultures, refer to B 37 – Investigation of blood cultures (for organisms other than *Mycobacterium* species) but microscopy, sub-culturing and further testing should be handled in accordance with the methods outlined in this SMI.

2.3 Adequate quantity and appropriate number of specimens[45]

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

3 Specimen transport and storage[23,24]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[45].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[45].

Note: Refrigerated storage of intravascular line tips does not significantly decrease the yield of culture[50].

4 Specimen processing/procedure[23,24]

4.1 Test selection

Culture techniques

Diagnosis of CR-BSI may be difficult due to the lack of clear clinical definitions. Definitive diagnosis can only be achieved if both the removed catheter tip / cannula cultured and blood cultures yield potentially pathogenic organisms in sufficient quantity[19]. Techniques that have been used to diagnose local or systemic infection associated with cannulae include[51]:

- semiquantitative and quantitative culture of cannula segments
- broth culture of cannula segments, particularly the tip
- staining of cannulae
- culture of blood aspirated through an intravascular cannula
- culture of the cannula hub
- culture of the cannula insertion site
- ultrasonication of cannulae

Semiquantitative method

Culture of the cannula surface is used to predict which are truly infected and likely to cause bloodstream infections. The terminal 4-5cm segment of the cannula (or as provided) is rolled over the entire surface of the agar plate back and forth several

times (4-5 times). The inoculated agar plate is incubated and the number of colonies on the plate is counted after incubation[7,52].

When culturing the external surface of the cannula tip, a threshold of >15 colonies of any organism is commonly accepted to predict cannula-related sepsis and is associated with bacteraemia in 10-14% of cases[7,53]. This threshold is based on the culture of a 4cm length. In practice, varying lengths of line are often received and interpretation should be made with care, and in conjunction with blood culture results. However, in practice this threshold may be too low where stringent removal precautions are not taken or there is a delay before processing. A threshold of >100 colonies may be more appropriate[52].

Multiple isolates present at >15 cfu are counted individually and their significance related to any blood culture isolate[7].

Quantitative method[54]

This method provides information on both the inner and outer surfaces of the cannula. A cut-off of 1000 cfu/mL is used as indicating sepsis[22,52]. The lumen result is reported to be a more reliable predictor of systemic infection where there is no evidence of localised exit site infection[52]. This method is labour intensive and is not recommended for routine use in this SMI. Both quantitative and semi-quantitative methods are equally effective in predicting absence of infection.

Enrichment method

The distal segment of the cannula is placed in enrichment broth, incubated for about 48hr and then organisms isolated on appropriate agar media. However, this does not distinguish among colonisation, infection or contamination of the cannula. It should be noted that the use of enrichment broth is not recommended except in certain settings eg such as if a source of candida fungaemia is suspected. However, its use is not recommended in this SMI.

Endoluminal brush

This has been reported as an accurate method of detecting catheter related sepsis without the need for catheter removal[55].

Rapid diagnostic methods

Staining the cannula (or an impression smear of the cannula) with Gram stain or acridine orange have been described and these provide information without the 24-48hr delay required for isolation of organisms[56,57].

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Stain an impression smear of the cannula or the isolate if clinically indicated either by Gram stain or Acridine Orange stain (refer to TP 39 – Staining procedures).

Note: Refer to manufacturers' instructions with respect to preparing smears from blood culture bottles.

4.5 Culture and investigation

Cannulae

Roll specimen across the agar surface several times (semiquantitative technique) to cover as much of the agar surface and external cannula surface as possible[52].

If >4cm is received, the distal end should be reduced to a 4cm length, prior to culture, by cutting with sterile scissors or scalpel.

Swabs

Inoculate agar plate with swab (refer to Q 5 – Inoculation of culture media in bacteriology). For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)‡
			Temp °C	Atmos	Time		
Cannula-related bacteraemia **Cannula-related infection**	Cannulae	Blood agar	35-37	5-10% CO_2	24-48hr	Daily	≥15 cfu per plate of any bacteria
Local cannula site infection	Swabs	Blood agar	35-37	5-10% CO_2	24-48hr	Daily	Coagulase negative staphylococci *S. aureus* including MRSA* *Corynebacterium* species Enterobacteriaceae Enterococci Pseudomonads Streptococci *Bacillus* species Yeasts#
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Optional media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Cannula-related fungaemia	Blood (after flagging positive on blood culture automated systems) Cannulae	Sabouraud agar supplemented with chloramphenicol and gentamicin or chromogenic agar[58,59]	28-30	Air	Up to 7d	2d and at 5d	Yeasts**
Cannula-related bacteraemia **Cannula-related infection**	Peripheral IV line tips (after there is a positive blood culture in the 7 days before or after the day the line tip is sent)[10,60]	Blood agar	35-37	5-10% CO_2	24-48hr	Daily	Coagulase negative staphylococci *S. aureus* including MRSA* *Corynebacterium* species Enterobacteriaceae Enterococci Pseudomonads Streptococci *Bacillus* species Yeasts#

* For more information on MRSA, refer to B 29 - Investigation of specimens for screening for MRSA.

** Some yeasts can grow at much higher temperatures such as *Malassezia* species which grow strictly at 30-35°C and not outside this range[61] .

Any cfu growth of yeasts is considered significant and should be reported.

‡ For appearance of relevant target organisms, refer to individual SMIs for organism identification.

Note: If automated monitoring systems are used, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.

Rapid test methods such as MALDI-TOF MS and NAATs should be performed according to manufacturers' instructions[26-28,62,63].

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

α-haemolytic streptococci	"α-haemolytic" level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
Coryneforms	"diphtheroids" level
Enterobacteriaceae	"coliforms" level
Enterococcus	genus level
Pseudomonads	"pseudomonads" level
S. aureus	species level
Yeasts	"yeasts" level

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Report organism seen.

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

5.2.1 Cannulae

Report the number of cfu of organism(s) isolated with an interpretative comment, eg ≥15 cfu may be associated with systemic cannula-related infection, or may represent superficial colonisation or contamination - refer to blood culture results

OR

Report absence of growth.

5.2.2 Swabs

Report the amount (eg heavy, moderate or scanty) of growth isolated with an interpretative comment relating to the presence of local infection

OR

Report the absence of growth.

5.2.3 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[64,65], or equivalent in the devolved administrations[66-69]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

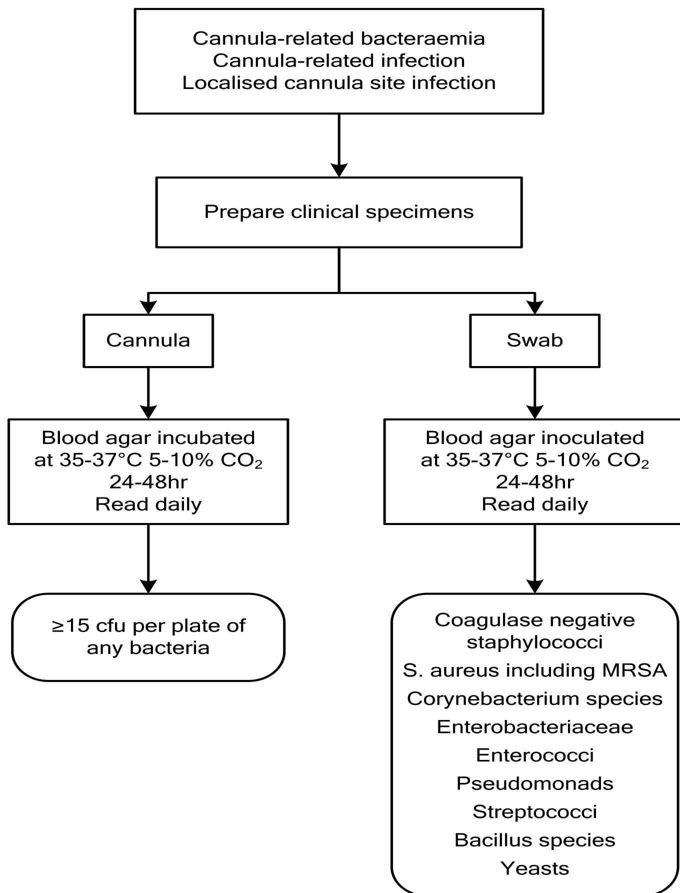
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[66,67], Wales[68] and Northern Ireland[69].

Appendix: Investigation of intravascular cannulae and associated specimens



Refer to 'B 37 - Investigation of blood cultures (for organisms other than Mycobacterium species)' for processing of blood culture.

This flowchart is for guidance only.

References

1. Garrison RN, Wilson MA. Intravenous and central catheter infections. Surg Clin North Am 1994;74:557-70.

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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 20 Investigation of intravascular cannulae and associated specimens

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

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Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 05/05/2015 – 02/06/2015

Version of document consulted on: B 20dp+

Proposal for changes

Comment number	1		
Date received	19/05/2015	**Lab name**	Oxford University Hospital NHS Trust Microbiology
Section			
Comment			
Consideration should be given to triage peripheral IV line tips in the following way. When IV line tips are received in the laboratory they are processed if there is a positive blood culture in the 7 days before or after the day the line tip is sent. If there is no positive blood culture the tip is refrigerated for 7 days and only cultured, if a blood culture becomes positive. This has been shown in a randomised study to reduce antibiotic use in ITU with no impact on morbidity, mortality or length of stay, see A. Perez-Parra et al Journal of Hospital Infection 77 (2011) 309-315. This policy has been shown to be acceptable and cost saving in the NHS, see Colston J et al.2013 J Hosp Infect. May; 84(1):77-80.			
Evidence			
Cost savings and clinical acceptability of an intravascular line tip culture triage policy. Colston J, Batchelor B, Bowler IC. 2013 J Hosp Infect. May;84(1):77-80 Prospective, randomised study of selective versus routine culture of vascular catheter tips: patient outcome, antibiotic use and laboratory workload Perez-Parra, M. Guembe, P. Martin-Rabadan, P. Munoz, A. Fernandez-Cruz, E. Bouza Journal of Hospital Infection 77 (2011) 309-315			
Health benefits			
A triage policy could save the NHS a large amount of money by reducing antibiotic use and reducing laboratory costs with improve quality of care for patients - see papers above.			
Recommended action	**ACCEPT** This has been updated accordingly.		

Comment number	2		
Date received	22/05/2015	**Lab name**	Salford Royal NHSFT
Section	4.1 & appendix		
Comment			
a. 4.1: the second sentence commencing 'definitive diagnosis........' Refers to diagnosis of CR-BSI and needs amending to include both positive cannula and			

blood cultures. b. Appendix. Two of the boxes refer to 'coagulase negative staphylococcus aureus'. These need amending to coagulase negative staphylococci (no italics). Otherwise this all looks good to me.

Recommended action	a. **ACCEPT** This section has been amended to include both cannulae and blood cultures b. **ACCEPT** The appendix has been amended to reflect the changes mentioned.

Comment number	3		
Date received	22/05/2015	**Lab name**	Truro
Section	Pgs 11, 14, 15, 19		
Comment			
a. Pg 11 - last paragraph, 1st line - should read MALDI b. Pg 14 – 1st line, immersed in sterile enrichment broth - No information regarding quantifying enrichment broth culture - how is this performed and related to infection? c. Pg 15 4.5.1 table - Standard media states blood agar for cannula-related bacteraemia/infection - ?broth d. Pg 19 2nd box down on the left - Blood agar incubated.... - What happened to the broth?			
Recommended action	a. **ACCEPT** This has been updated accordingly. b. **ACCEPT** This section has been updated with appropriate information. The reason as to why the enrichment broth is not recommended for use in this document is mentioned under section 4.1 subheading – Enrichment method. c. **NONE** This document does not recommend the use of the enrichment broth for culture except in instances when a candida infection is under investigation. d. **NONE** Not recommended in this document.		

<table>
<tr><td>Comment number</td><td>4</td><td colspan="2"></td></tr>
<tr><td>Date received</td><td>28/05/2015</td><td>Lab name</td><td>IBMS</td></tr>
<tr><td>Section</td><td colspan="3">Various</td></tr>
<tr><td colspan="4">Comment</td></tr>
<tr><td colspan="4">
a. Introduction
i. The third paragraph makes reference to the EPIC guidelines in relation to prevention of HCAIs associated with use of central venous catheters. It is suggested that reference (2) is superseded by the updated version of these guidelines which is EPIC3 published in Dec 2013. H.P. Loveday et al. Journal of Hospital Infection 86S1 (2014) S1-S70
ii. Also in the third paragraph CR-BSI is briefly discussed. It might be useful to reference this to the Matching Michigan project ‘Matching Michigan’: a 2-year stepped interventional programme to minimise central venous catheter-blood stream infections in intensive care units in England. BMJ Qual Saf doi:10.1136/bmjqs-2012-001325
b. Specimen Transport and Storage
The text specifies that ‘Specimens should be transported and received in the lab within one working day of collection and processed as soon as possible. Requirements of individual testing labs should be referred to reference 45’.
Reference 45 depicts DH prevention and treatment of tuberculosis. Is this a correct citation for the IV cannulae SMI?
c. Specimen processing /procedure
Semi-quantitative method
Page 14, second paragraph, reference 48 dates back to 1977. It is queried whether it is appropriate to quote a reference which is nearly 40yrs old in relation to the measuring criteria to determine if resultant growth is clinically significant ie > 15 CFUs.
In addition, there doesn’t appear to be a reference for the suggestion of a higher threshold ie >100 CFUs.
d. General comment
It has been noted that some laboratories receive additional samples which are treated in a very similar way to IV cannulae. For example pacing wires undergo enrichment culture similar to that described in Section 4 Specimen Processing/Procedure. Skin swabs from the entry site of drivelines associated with Ventricular Assist Devices are processed in the same way as swabs from IV access sites. Should these sample types be listed in the introduction or are they covered in other SMIs?
</td></tr>
<tr><td>Recommended action</td><td colspan="3">
a. i) ACCEPT
This has been updated accordingly.
ii) ACCEPT
This has been updated accordingly.
</td></tr>
</table>

	b. **ACCEPT** This has been updated accordingly. c. i) **NONE** The reference by MAKI et al 1977 although quite old, this is the original paper that describes semi-quantitative culture method and all the recent journals refer to this hence the reason why it was used. ii) **ACCEPT** This has been updated accordingly with a reference added to this section. d. **NONE** Information on the exclusion of pacing wires has been added into the scope of document to make it clear.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 3			
Date received	19/05/2015	**Lab name**	General Practitioner
Date received	19/05/2015	**Lab name**	Microbiology Department Antrim Area Hospital
Date received	19/05/2015	**Lab name**	NMC

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UK Standards for Microbiology Investigations

Investigation of Cerebrospinal Fluid Shunts

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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 22 | Issue no: 6.1 | Issue date: 12.06.15 | Page: 1 of 20

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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

SCOPE 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 9

1 SAFETY CONSIDERATIONS 11

2 SPECIMEN COLLECTION 11

3 SPECIMEN TRANSPORT AND STORAGE 12

4 SPECIMEN PROCESSING/PROCEDURE 12

5 REPORTING PROCEDURE 15

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 16

APPENDIX: INVESTIGATION OF CEREBROSPINAL FLUID SHUNTS 17

REFERENCES 18

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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	9/12.06.15
Issue no. discarded.	6
Insert Issue no.	6.1
Section(s) involved	**Amendment**
Appendix.	Incubation time for fastidious anaerobe agar changed to 10 days.

Amendment No/Date.	8/18.05.15
Issue no. discarded.	5.2
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Types of specimen.	List of specimen types compressed.
Introduction.	Contents displayed in bullet points rearranged in to prevalence order.
Technical information/limitations.	Section expanded.
Section 2.5.3.	Tables amended to include specimen type.
Appendix.	Flowchart presentation amended to look more similar to the contents of the table.
References.	References reviewed and updated.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

Public Health England. (2015). Investigation of Cerebrospinal Fluid Shunts. UK Standards for Microbiology Investigations. B 22 Issue 6. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Cerebrospinal fluid shunt, shunt tubing, pus

Scope

This SMI describes the processing and bacteriological investigation of cerebrospinal fluid shunts.

This SMI should be used in conjunction with other SMIs.

Introduction

Hydrocephalus

Hydrocephalus is a condition caused by the accumulation of excess cerebrospinal fluid (CSF) within the cerebral ventricular system[1]. It occurs in both adults and children. If untreated, the prognosis is poor. It may be classified as[2]:

- communicating (no block between the ventricles and subarachnoid space)
- non-communicating (a block is present between the ventricles and subarachnoid space)

The common causes of hydrocephalus are an obstruction of the flow of CSF or a failure to absorb it, resulting from, in order of prevalence:

- major developmental abnormalities such as spina bifida
- meningitis
- perinatal haemorrhage
- trauma
- tumours, especially in the posterior fossa
- normal pressure hydrocephalus, a form of reversible dementia affecting the elderly
- overproduction of CSF

Treatment for hydrocephalus involves diverting CSF from the ventricular system to another compartment where it can be absorbed directly or indirectly into the bloodstream. This is done by means of a shunt. There is a risk of infection at the initial shunt insertion and at each subsequent insertion, and shunts may also be infected at other times.

Shunts

Shunts consist of drainage tubes incorporating one or more valves to control the direction and rate of CSF flow[2]. The devices may also incorporate a reservoir. There are two main types of shunt[2]:

- ventriculo-atrial (VA) shunts are used to drain CSF from the ventricle to the right atrium
- ventriculo-peritoneal (VP) shunts are more commonly used in contemporary neurosurgical practice; in these, the route of drainage is from the ventricle to the peritoneal cavity

Shunt replacement is necessary from time to time due to growth of the recipient or to mechanical obstruction or infection of the device.

If a shunt has to be removed because of infection, CSF drainage has to be maintained. This can be achieved by means of an implanted reservoir (which can be tapped as required) or by an external ventricular drain (EVD). These systems allow instillation of intrathecal antibiotics to treat ventriculitis before implantation of a new shunt. They may themselves become secondarily infected. These systems are also used to relieve hydrocephalus in the short term in patients who may not require a permanent shunt.

CSF shunts become infected by the following routes, in order of significance:

- organisms directly colonise the shunt, usually at the time of surgery
- organisms travel along the shunt by retrograde spread
- organisms reach the CSF and the shunt via haematogenous spread

Indicators of infection differ according to the type of shunt. For instance:

- signs of VA and VP shunt malfunction (and/or meningitis) include symptoms such as headaches, vomiting, drowsiness and decreased level of consciousness, with or without fever
- infected VA shunts discharge organisms directly into the right cardiac atrium. This gives rise to intermittent fevers and signs of bacteraemia. Rarely, shunt nephritis may occur a long time (sometimes several years) after initial shunt surgery. It is a result of the formation and deposition of immune complexes on the glomeruli basement membranes, and is seen only in VA shunts
- infected VP shunts discharge organisms directly into the peritoneal cavity, or may become distally infected without causing meningitis. Abdominal pain as a result of local inflammation may occur, as may local erythema over the shunt track. Rarely, the distal portion of the shunt may perforate the bowel, leading to peritonitis and abscess formation. Sometimes in such cases, polymicrobial ventriculitis, including anaerobes, can be found

Peritoneal fluid may be sent for culture if there is evidence of peritoneal inflammation. Mixed infections, particularly if colonic anaerobic bacteria are present, suggest bowel perforation.

Shunts which are removed should be sent for culture. Shunt infections may be confirmed by recovering the organism from blood cultures (see B 37- Investigation of blood cultures for organisms other than Mycobacterium species), CSF (see B 27 - Investigation of cerebrospinal fluid), shunt tubing, valves or a combination of these. It should be remembered that CSF microscopy may be unremarkable in shunt infection.

Intraventricular catheterisation (or external ventricular drainage) is used to monitor intracranial pressure in a variety of neurological and neurosurgical disorders, especially trauma[3]. Catheters used for this purpose may also be sent for culture (see

B 20 - Investigation of intravascular cannulae and associated specimens). Recently intracranial pressure 'bolts' have been introduced: this reduces the need for more invasive ventricular catheterisation in many patients.

Organisms isolated from CSF shunts and ventricular catheters include the following with coagulase negative staphylococci being the most common[2,4]:

- coagulase negative staphylococci
- *Staphylococcus aureus*
- enterobacteriaceae
- coryneforms and *Propionibacterium* species
- enterococci
- pseudomonads
- streptococci
- yeasts
- *Mycobacterium* species

The staphylococci amount for 60-85% of infections. *P. acnes* has been found in about 10% of shunt infections but usually only after prolonged anaerobic incubation. External ventricular drainage infections are also caused mainly by staphylococci but there is often a larger proportion of Gram negative bacteria including *Acinetobacter* species.

Organisms which may be isolated but less frequently include anaerobes and fungi other than yeasts[5-8].

The usual community-acquired bacteria (*Haemophilus influenza, Neisseria meningitidis* and *Streptococcus pneumoniae*, can cause meningitis in patients with shunts but they do not usually cause shunt infections, and the meningitis should be treated without removal of the shunt unless it is malfunctioning.

Biofilms have been shown to be a problem when dealing with shunt infections and can cause delays in the effect of treatment[9,10]. CSF results are of questionable value when biofilm infections are involved. Some bacteria are more prone to form biofilms than others[10,11].

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance

therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[12,13]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

1 Safety considerations[12-28]

1.1 Specimen collection, transport and storage[12-17]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[12-28]

Containment Level 2 unless infection with a) *N. meningitidis,* b) a Hazard group 3 organism or c) TSE is suspected.

a) Although *N. meningitidis* is in Hazard group 2, suspected and known isolates of *N. meningitidis* should always be handled in a microbiological safety cabinet. Sometimes the nature of the work may dictate that full containment level 3 conditions should be used eg for the propagation of *N. meningitidis* in order to comply with COSHH 2004 Schedule 3 (4e).

b) Where Hazard Group 3 *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

c) If TSE is suspected, laboratory policies that take into account the local risk assessments may dictate that the use of a microbiological safety cabinet should be used when dispensing the specimen. Check recent ACDP guidelines on this area.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[20].

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65-75°C), under the hood, until dry. Then place in a rack or other suitable holder.

Note: Heat-fixing may not kill all *Mycobacterium* species[29]. Slides should be handled carefully.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Cerebrospinal fluid shunt, shunt tubing, pus

2.2 Optimal time of specimen collection[30]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[30].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

When a shunt is removed all three portions should be sent in separate microbiologically approved containers of the appropriate size[13]. This will include the proximal catheter, a valve or reservoir, and a distal catheter[31]. CSF is usually obtained from the shunt reservoir and sent concurrently for investigation (see B 27 - Investigation of cerebrospinal fluid).

2.3 Adequate quantity and appropriate number of specimens[30]

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport and storage[12,13]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[30].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[30].

4 Specimen processing/procedure[12,13]

4.1 Test selection

Microscopy and culture should be carried out as outlined below.

4.2 Appearance

Look for pus on external surface.

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

If the whole shunt is received intact, separate and process each portion separately.

If shunt tubing is received, cut a 5cm length aseptically from each end.

If CSF is visible in the shunt tubing or reservoir, aspirate it with a sterile pipette and process accordingly (see B 27 - Investigation of cerebrospinal fluid). It is important to record the section from which the CSF is withdrawn to assist in deciding the aetiology of the infection and significance of isolates obtained.

If no CSF in the reservoir flush the tubing with sterile saline and collect fluid in a CE Marked leak proof container in a sealed plastic bag. In the absence of CSF sample this can be used.

4.3.2 Specimen processing

Pus

Swab any visible pus on the surface of the tubing[31].

(Process separately from the flushed tubing - see below).

Inoculate each agar plate with swab (see Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Tubing

Using a sterile pipette inoculate each agar plate with the uncentrifuged, flushed saline (see Q 5 - Inoculation of culture media for bacteriology).

Note: The use of broth medium for processing shunt tubing can lead to false positive results and is not recommended[31].

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.4 Microscopy

4.4.1 Standard

TP 39 - Staining procedures

Fluids

Any fluid aspirated from shunt tubing or other component is treated as CSF (see B 27 - Investigation of cerebrospinal fluid).

Pus (from external surfaces)

Prepare a thin smear on a clean microscope slide for Gram staining.

4.4.2 Supplementary

N/A

4.5 Culture and investigation

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Shunt infection	Cerebrospinal fluid shunt, shunt tubing, pus	Chocolate agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
		Blood agar	35-37	5-10% CO_2	40-48hr	daily	
		Fastidious anaerobe agar	35-37	anaerobic	10d[32]	≥40hr, 5d and at 10 days if you have an anaerobic cabinet otherwise at 10 days	Anaerobes
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Optional media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
If fungi are seen on microscopy	Cerebrospinal fluid shunt, shunt tubing, pus	Sabouraud agar	35-37	air	40-48hr	≥40hr*	Yeasts Fungi

*incubation may be extended to 10 days; in such cases plates should be read at ≥40hr and then left in the incubator/cabinet until day 10. If using jars then the first reading should be at 5 days. Certain opportunistic pathogens will require extended incubation. Laboratories should take precautions to stop plates drying out.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	species level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
All other organisms	species level if in pure culture or clinically indicated

Organisms may be further identified if this is clinically or epidemiologically indicated.

It may be useful to store coagulase negative staphylococci in case it is necessary to distinguish re-infections from relapsed infections.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Report microscopy on the CSF (see B 27 - Investigation of cerebrospinal fluid), or pus from external surface.

5.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

5.2 Culture

Report organisms isolated or

Report absence of growth.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[33,34] or equivalent in the devolved administrations[35-38]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England(PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

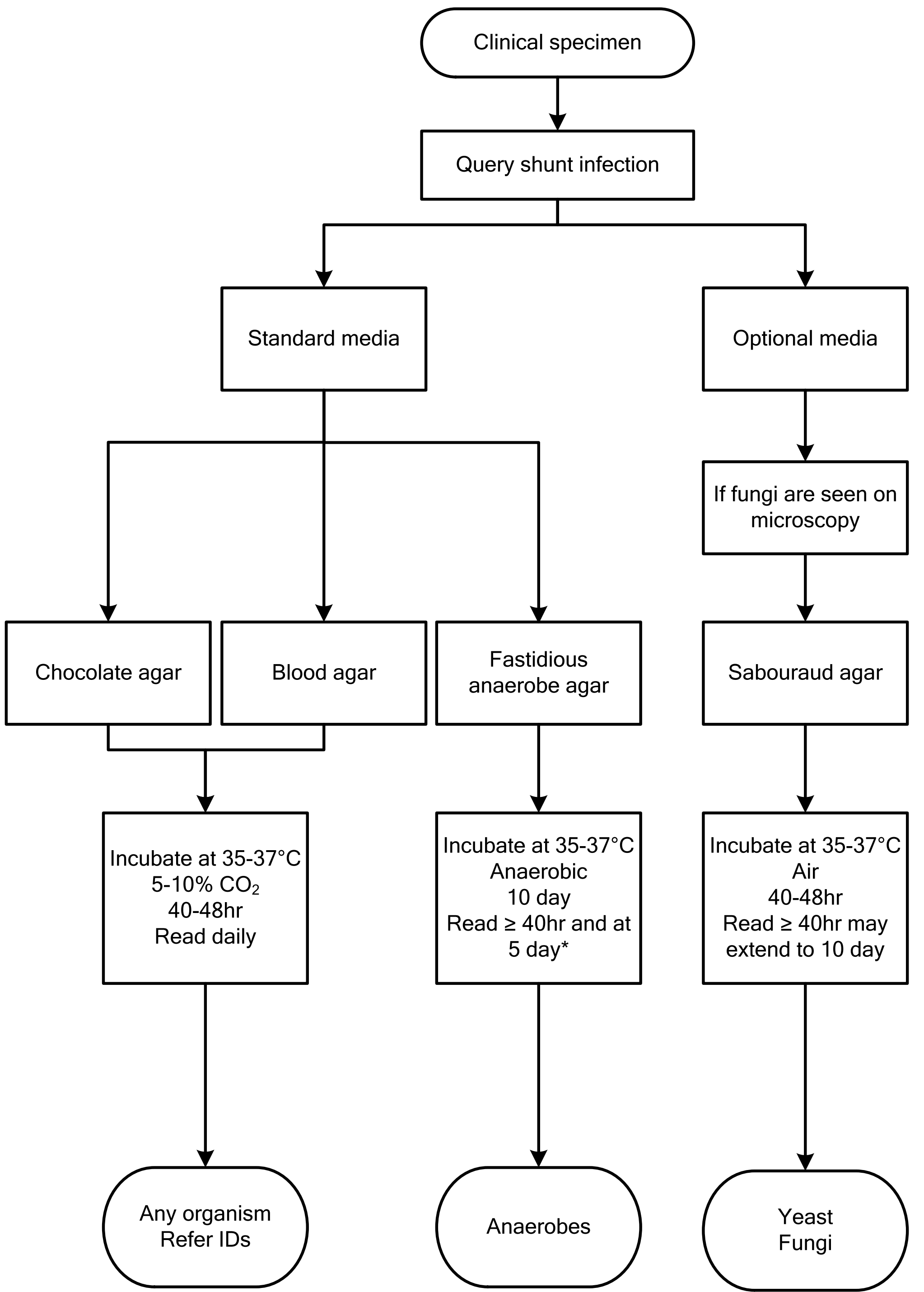
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[35,36], Wales[37] and Northern Ireland[38].

Appendix: Investigation of cerebrospinal fluid shunts



*if using an anaerobic incubator/cabinet. Otherwise the first reading should be at 5 days

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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 22 Investigation of Cerebrospinal Fluid Shunts

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation 14.05.14 – 02.06.14

Version of document consulted on – B 22dj+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	02/06/2014	**Professional Body**	UK CMN
Section	4.5.1 & Appendix		
Comment			
a. Section 4.5.1 If fungi seen on microscopy incubation time should be extended up to 10 days b. Appendix Should extend incubation of Sab plates as fungi have been seen on microscopy to 10 days			
Financial Barriers			
No.			
Health Benefits			
No.			
Recommended Action	**ACCEPT**		

COMMENTS RECEIVED OUTSIDE OF CONSULTATIONS

Comment Number	1		
Date Received	03/01/2013	**Lab Name**	Southampton General Hospital
Section	Table headed - Clinical details/conditions conditions		
Comment			
There is no comment sheet for this but it still looks as though it's under review SOP numbers B 22 and B 27 both concerning CSF and CSF shunts. I have just had one of our consultants ask why we weren't following these SOPs. We have found conflicting and confusing information. For the investigation of CSF culture the incubation times states 7 to 14 days but only states to read at >40hrs and at 5 days with no mention of 14 day reading anywhere. Please could this be clarified for us. We have only followed the reading and reporting at 5 days but will change as soon as this is understood.			
Recommended Action	**ACCEPT**		

	This has now been streamlined in all CSF.

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 5			
Date Received	02/08/2013	**Lab Name**	Royal Cornwall Hospital Truro, Cornwall
Date Received	15/05/2014	**Lab Name**	Nottingham NUH
Date Received	20/05/2014	**Lab Name**	Royal Oldham Hospital
Date Received	30/05/2014	**Lab Name**	Truro, Cornwall
Date Received	31/05/2014	**Lab Name**	Italian Microbiology Laboratory

UK Standards for Microbiology Investigations

Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid

NICE accredited
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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 25 | Issue no: 6 | Issue date: 20.02.15 | Page: 1 of 21

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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

SCOPE 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 11

2 SPECIMEN COLLECTION 11

3 SPECIMEN TRANSPORT AND STORAGE 12

4 SPECIMEN PROCESSING/PROCEDURE 12

5 REPORTING PROCEDURE 16

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 16

APPENDIX: INVESTIGATION OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS FLUID 18

REFERENCES 19

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	10/20.02.15
Issue no. discarded.	5.3
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Scientific content reviewed and no substantive changes made.
Table and Appendix.	Antimicrobial substance testing removed.
References.	References reviewed and updated.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested Citation for this Document

Public Health England. (2015). Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid. UK Standards for Microbiology Investigations. B 25 Issue 6. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of Document

Type of Specimen

Continuous ambulatory peritoneal dialysis (CAPD) fluid

Scope

This SMI describes the processing and microbiological investigation of continuous ambulatory peritoneal dialysis fluid.

This SMI should be used in conjunction with other SMIs.

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is used as an alternative to haemodialysis for the management of patients with end-stage renal failure. In this procedure the patient's own peritoneal membrane is used to dialyse waste products from the patient's blood. CAPD encompasses a closed system of commercially prepared sterile dialysate fluid in a bag, connected by silastic tubing to a Tenchkoff catheter which leads the fluid in and out of the peritoneal cavity. This achieves hyperosmolar ultrafiltration across the peritoneal membrane. Usually 1-2 litres of dialysate is infused every 6 hours and the effluent drainage is collected by gravity into the empty dialysate bag at the end of each cycle.

CAPD has many advantages over haemodialysis. There is no requirement for vascular access or for specialised equipment in the home. Moreover, patients are more mobile and independent, and are able to carry out the bag changes without assistance.

However, peritonitis is a frequent complication of CAPD[1]. Most CAPD infections arise from direct contamination of the catheter. On rare occasions infections may originate from an intra-abdominal focus such as diverticulitis[1]. The vast majority of CAPD infections are unimicrobial. Infection may involve the catheter exit site, subcutaneous tunnel, or the peritoneum.

Clinical manifestations of infection in patients undergoing CAPD include:

- Cloudy dialysis effluent
- Abdominal pain and tenderness
- Fever
- Nausea
- Vomiting
- Chills
- Erythema at the catheter site
- Discharge at the catheter site
- Catheter malfunction and drainage problems

Diagnosis of CAPD Peritonitis

This requires high quality microbiological facilities and close liaison between the clinician and microbiology department. Clinical diagnosis is usually based on the presence of at least two of the following criteria:

- Cloudy dialysate effluent
- Symptoms of peritonitis
- Positive culture and/or Gram stain of peritoneal fluid

Microscopy

Cloudiness generally represents a white blood cell (WBC) count of >100 x 10^6 per litre[1]. The presence of chyle, fibrin or blood may also cause turbidity, so microscopy is essential to confirm the presence of WBCs. Fluids with WBC counts of 50 - 100 x 10^6 per litre may be macroscopically clear.

The presence of >100 WBC x 10^6 per litre correlates closely with infection[1], although many false negative culture results have been reported. This is less likely with WBC counts of 500 x 10^6 per litre or above[1]. However, low WBC counts of <100 x10^6 per litre may be associated with the early stages of infection[1].

In most infected dialysates polymorphonuclear leucocytes (PMNs) predominate[1,2]. Routine differentiation of WBC morphology is of little diagnostic value[3]. However, >100 x10^6 eosinophils per litre can indicate allergic reaction, and occur in patients with the aetiologically unclear "eosinophilic peritonitis", with fungal peritonitis, or in those who have received intraperitoneal or systemic antibiotics[1].

There is no correlation between the WBC count and the number of bacteria present in dialysis effluent. Despite large numbers of WBCs, organisms may not be visible or they may be present in low numbers because of their sequestration within the phagocytes. Hence sensitivity of Gram stain is low (about 50%) except where there are large numbers of organisms present[1].

Culture

Recovery of organisms on culture may be difficult therefore the UK Renal Association recommends that the negative peritoneal fluid culture rates in patients with clinical peritonitis should be less than 10% although others have disputed this figure as too low. Recovery of organisms in culture can be increased by lysis of WBCs which releases sequestered organisms[4]. Various methods for lysing WBCs with varying degrees of success in recovering the organisms have been reported and these are covered in more detail below:

- Water lysis is recommended to minimise the problem of toxicity to delicate organisms that was encountered when lytic agents such as bile salts or Triton-X were used and reduces the risk of contamination found with broth methods[5]
- A lysis-centrifugation method will yield a positive culture rate of about 85%[2,6,7]. There is currently no satisfactory culture method for detecting the cause of the remaining 15% culture negative, clinically infected patients[3]
- Filtration of unlysed CAPD effluent and enrichment methods of culture are less sensitive than centrifugation after white cell lysis; both are more sensitive than centrifugation without white cell lysis[5]

Coagulase negative staphylococci are the commonest causes of CAPD peritonitis, but also the commonest laboratory contaminants[3].

Direct inoculation of blood culture bottles by CAPD staff is popular[8-11]. This method of culture may be useful for the early detection of infection where there will be a delay in receipt of the CAPD dialysate in the laboratory[12]. A protocol to minimise ward-based contamination during sampling and inoculation of blood culture bottles should be agreed with clinical staff, similar to that used for regular blood cultures. For patients on treatment, blood culture bottles containing antimicrobial removal resins are reported as having a higher isolation rate than those without[9,12]. The bottles should be accompanied by a separate specimen for microscopy and direct culture. Inclusion of direct culture on blood-containing media is recommended to allow recovery of fastidious micro-organisms that will not grow in blood culture bottles that do not contain blood.

Organisms most commonly isolated from CAPD dialysate are[1,10]:

- Coagulase negative staphylococci
- *Staphylococcus aureus*
- Enterobacteriaceae
- Pseudomonads
- *Acinetobacter* species
- Enterococci
- Streptococci
- *Corynebacterium* species

This list is not exhaustive, and a wide range of unusual and fastidious organisms have been isolated from CAPD dialysate[1].

Anaerobes are a relatively uncommon cause of CAPD peritonitis, but do occur as a result of bowel perforation (eg in diverticulitis).

Mycobacterium species - if routine cultures are negative and abnormal dialysate findings persist after treatment of presumed or documented bacterial peritonitis, evidence of infection with *M. tuberculosis* should be sought particularly if tuberculosis is endemic in the patients country of origin[13-15].

Non-tuberculous *Mycobacterium* species are identified, although rarely, as causes of infective peritonitis in patients undergoing CAPD.

Fungal peritonitis occurs with the most common isolates being *Candida* species[16,17]. *Cryptococcus neoformans* may be isolated, although rarely[18].

Polymicrobial infections have been reported and should be considered in certain patients[2].

16S rDNA PCR and other non-culture detection methods may be a useful diagnostic tool in addition to culture for certain cases[19].

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[20,21]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

1 Safety Considerations[20-36]

1.1 Specimen Collection, Transport and Storage[20-25]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Large volumes or whole dialysate bags may require special transportation according to local protocols. They should be transported in rigid, leakproof outer containers.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[20-36]

Containment Level 2.

Where Hazard Group 3 *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[28].

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65-75°C), under the hood, until dry. Then place in a rack or other suitable holder.

Note: Heat-fixing may not kill all *Mycobacterium* species[37]. Slides should be handled carefully.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

Continuous ambulatory peritoneal dialysis (CAPD) fluid

2.2 Optimal Time and Method of Collection[38]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[38].

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[39-43].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Receipt of the whole dialysate bag is preferable so that sampling under controlled laboratory conditions may be performed.

Where safe transport and receipt of the whole bag is considered impractical, withdraw fluid aseptically from the injection port of the plastic dialysate bag with a sterile needle and syringe and transfer to a microbiologically approved container[21,44].

If blood culture bottles are used they should be inoculated aseptically with 5-10mL of dialysate according to local protocol agreed between the laboratory and clinical staff.

2.3 Adequate Quantity and Appropriate Number of Specimens[38]

A volume of 10-50mL of fluid is considered suitable. Blood culture bottles may also be inoculated and submitted to the laboratory in addition to the pure sample.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage[20,21]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[38].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[38].

4 Specimen Processing/Procedure[20,21]

4.1 Test Selection

Microscopy and culture for *Mycobacterium* species if routine bacteriology cultures are negative and abnormal dialysate findings persist - see B 40 - Investigation of Specimens for *Mycobacterium* species.

4.2 Appearance

Describe as clear or cloudy fluid.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

Standard

Water-lysis method

Centrifuge 25mL of dialysate at 1500 x g for 5min.

Discard the supernatant or transfer to another microbiologically approved container for further testing if required leaving approximately 0.5mL deposit[21].

Resuspend the centrifuged deposit in 10mL of sterile distilled water by vigorous shaking for 30sec[45].

Centrifuge at 1500 x g for 5min.

Discard the supernatant, leaving approximately 0.5mL.

Resuspend the centrifuged deposit in the remaining fluid.

Blood Culture Bottles

If blood culture bottles are used they should be inoculated aseptically with 5-10mL of dialysate[10].

Supplementary

Mycobacterium species - B 40 - Investigation of Specimens for *Mycobacterium* species.

4.4 Microscopy

4.4.1 Standard

Cell count

Perform total cell count on uncentrifuged specimen[3].

4.4.2 Supplementary

Gram stain

Place one drop of centrifuged deposit (see Section 4.5.1) with a sterile pipette on to a clean microscope slide[3].

Spread this with a sterile loop to make a thin smear for Gram staining.

Differential leucocyte counts for eosinophils (for total counts of >100 x 10^6 WBC/L)

Prepare a slide from the centrifuged deposit as for Gram stain, but allow to air dry because heat fixation distorts the cellular morphology. Fix in alcohol and stain with a stain suitable for WBC differentiation.

Patients with low WBC counts have been shown to be culture negative[10].

Microscopy for Mycobacterium species - see B 40 - Investigation of Specimens for *Mycobacterium* species.

4.5 Culture and Investigation

Inoculate each agar plate with centrifuged deposit using a sterile pipette (Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp. °C	Atmos.	Time		
All conditions	CAPD	Blood agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
		Blood agar	28-30	air	40-48hr	daily	Psychrophilic pseudomonads
		Fastidious anaerobe agar	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp. °C	**Atmos.**	**Time**		
Peritonitis (microscopy suggestive of mixed infection)	CAPD	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
Clinical details/ conditions	**Specimen**	**Optional media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp. °C	**Atmos.**	**Time**		
Peritonitis (microscopy suggestive of mixed infection)	CAPD	Staph/strep selective agar	35-37	air	40-48hr	daily	*S. aureus* Streptococci
		CLED/MacConkey agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae
		Sabouraud agar	35-37	air	40-48hr*	≥40hr	Fungi
If bottles received or for enrichment. Any suspected infection	CAPD	Enriched culture eg Blood culture bottles subcultured to: Blood agar Fastidious anaerobe agar	35-37 35-37 35-37	air 5-10% CO_2 anaerobic	40-48hr 40-48hr 40-48hr	continuous monitoring (minimum 40-48hr) Daily Terminal subculture if appropriate ≥40hr	Any organism
Other organisms for consideration – *Mycobacterium* species (B 40)							
*incubation may be extended to 5 d if clinically indicated; in such cases plates should be read at ≥40hr and then left in the incubator/cabinet until day 5. Certain opportunistic pathogens will require extended incubation.							

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4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Enterococcus	genus level
Coagulase negative staphylococci	"coagulase negative" level
All other organisms	species level

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: Any organism considered to be a contaminant may not require identification to species level.

Mycobacterium species see B 40 - Investigation of Specimens for *Mycobacterium* species.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

Cell count

Report numbers of WBCs x 10^6 per litre.

Gram stain (if performed)

Report on organisms detected.

Differential leucocyte count (if performed)

Report numbers of eosinophils x 10^6 per litre.

Microscopy for *Mycobacterium* species – see B 40 - Investigation of Specimens for *Mycobacterium* species.

Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16–72hr.

5.2 Culture

Report the organisms isolated **or**

Report absence of growth.

Also, report results of supplementary investigations.

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically.

Written report, 16–72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations: *Mycobacterium* species - see B 40 - Investigation of Specimens for *Mycobacterium* species.

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[46,47] or Equivalent in the Devolved Administrations[48-51]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been

notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

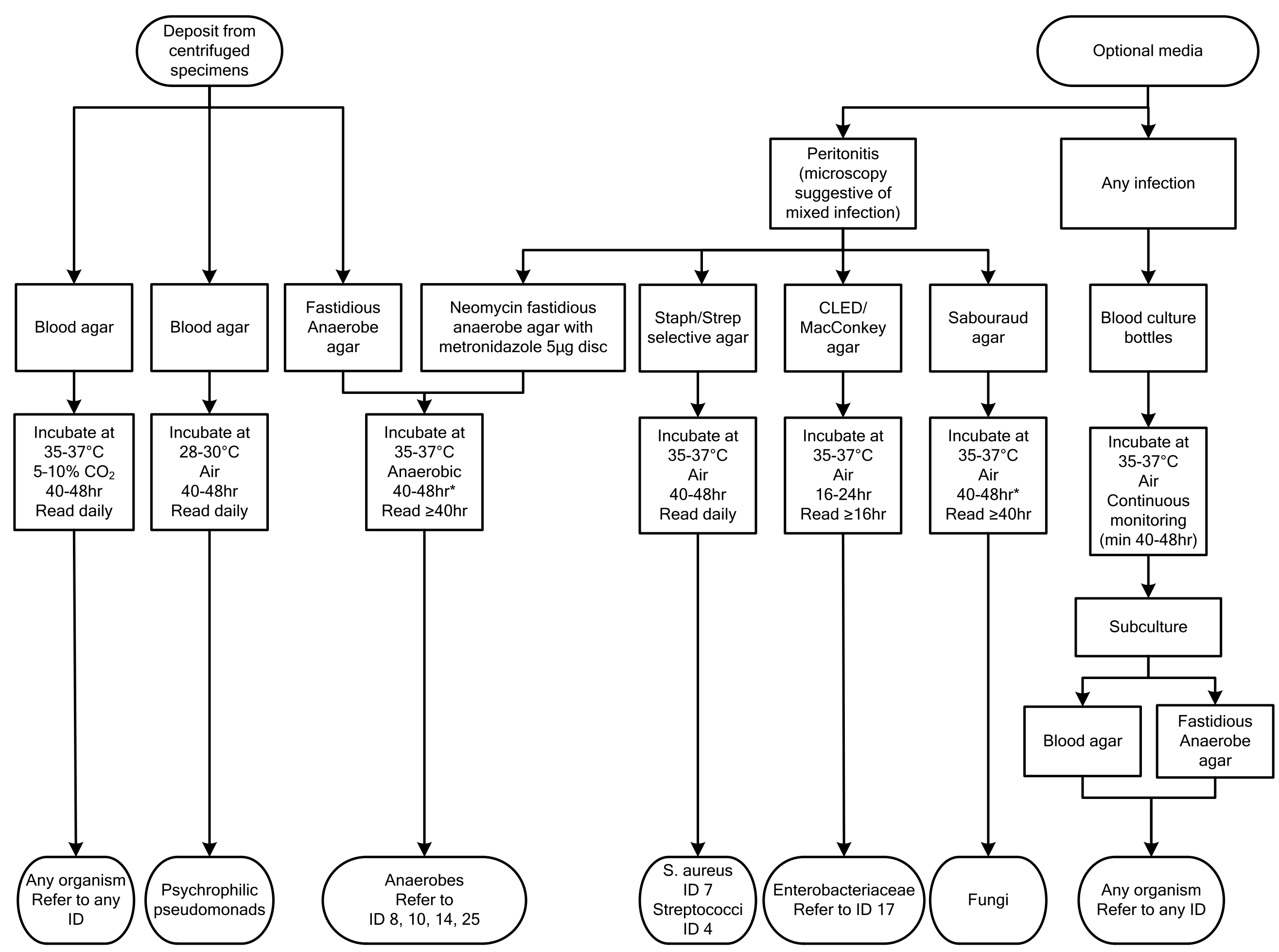
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[48,49], Wales[50] and Northern Ireland[51].

Appendix: Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid



* Incubation may be extended to 5d if clinically indicated; in such cases plates should be read at ≥40hr and then left in the incubator / cabinet until day 5.

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UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 25 Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 10/05/2013 – 02/08/2013

Version of document consulted on: B 25de+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	04/06/2013	**Lab Name**	Microbiology Laboratory University Hospital Birmingham
Section	Various		
Comment			
a. Section 2.4.2: Clarification and detail on the method to quantify the eosinophil count would be helpful. b. Is this expected to be carried out in a Microbiology Laboratory, Haematology or Cytopathology? c. Section 2.5.3: Is inoculation in blood culture bottles acceptable instead of BHI? Should terminal cultures be recommended in this case? d. Section 2.6.1: Is identification of coagulase negative staphylococci to 'coagulase-negative staphylococcus level sufficient, or should identification to species level be recommended, which would be particularly useful in cases or relapse/recurrence? e. More advice on the use of 16S PCR would be useful.			
Evidence			
Alfa MJ et al. JClin Micro 1997			
Recommended Action	a. **NONE** A decision on which stain is suitable needs to be made at a local level. b. **NONE** This decision needs to be taken at the local level c. **ACCEPT** Document has been amended. d. **ACCEPT** This part of the UK SMI has been amended for clarity. e. **ACCEPT** A recommendation to use non culture techniques where appropriate has been inserted with a reference.		

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Investigation of Fluids from Normally Sterile Sites

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 26 | Issue no: 6.2 | Issue date: 03.10.18 | Page: 1 of 24

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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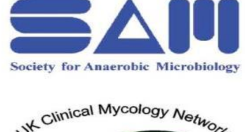

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Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 6

SCOPE OF DOCUMENT 8

SCOPE 8

INTRODUCTION 8

TECHNICAL INFORMATION/LIMITATIONS 11

1 SAFETY CONSIDERATIONS 12

2 SPECIMEN COLLECTION 12

3 SPECIMEN TRANSPORT AND STORAGE 13

4 SPECIMEN PROCESSING/PROCEDURE 13

5 REPORTING PROCEDURE 18

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 19

APPENDIX: INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES 21

REFERENCES 22

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	11/03.10.18
Issue no. discarded.	6.1
Insert Issue no.	6.2
Section(s) involved	**Amendment**
4.5.1 Culture media, conditions and organisms.	Asterix has been added to the incubation time for “blood agar” and “chocolate agar” to denote what is in the footnote. Spelling of *Nocardia* corrected where it appeared as Norcardia.

Amendment No/Date.	10/08.03.17
Issue no. discarded.	6
Insert Issue no.	6.1
Section(s) involved	**Amendment**
4.5.1 Culture media, conditions and organisms.	Enrichment broth has been added to the table.

Amendment No/Date.	9/15.06.15
Issue no. discarded.	5.2
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks changed to gov.uk.
Page 2.	Updated logos added.
Title.	Title amended.
Introduction.	Reviewed and streamlined.

3.1 Optimal transport and storage conditions.	Parameters set for each stage.
4.4 Microscopy.	Total white cell count section amended to now include the use of blood cell analysers.
4.5 Culture and investigation.	Use of blood culture bottles now a recognised method.
References.	References reviewed and updated.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (2018). Investigation of Fluids from Normally Sterile Sites. UK Standards for Microbiology Investigations. B 26 Issue 6.2. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Amniotic fluid, pericardial fluid, peritoneal fluid (ascites), pleural fluid, synovial (joint) fluid, bursa fluid

Blood, cerebrospinal fluid, continuous ambulatory peritoneal dialysis (CAPD) fluid, Pouch of Douglas fluid, bile and urine are dealt with in the following respectively:

- B 37 – Investigation of blood cultures (for organisms other than *Mycobacterium* species)
- B 27 – Investigation of cerebrospinal fluid
- B 25 – Investigation of continuous ambulatory peritoneal dialysis fluid
- B 28 – Investigation of genital tract specimens
- B 15 – Investigation of bile and
- B 41 – Investigation of urine

Scope

This SMI describes the examination of fluids for the detection and recovery of the causative organisms of infections of normally sterile sites (other than those listed above).

This SMI should be used in conjunction with other SMIs.

Introduction

The detection of organisms in fluids that are normally sterile indicates significant infection, which can be life-threatening.

Blood cultures may also be positive with the same infecting organism, and occasionally may be positive when culture of the fluid fails to reveal the organism. It is also possible to use blood culture bottles for the culture of sterile fluids[1].

Amnionitis[2]

Amnionitis means inflammation of the amnion, the innermost of the two membranes that form the fetal sac, enclosing the fetus and the amniotic fluid. In cases of prolonged rupture of the membranes, the amniotic fluid may become contaminated with vaginal flora. If amnionitis is confirmed during labour, infants are delivered immediately depending on fetus age due to the risk of infection to the mother and the foetus. Amnionitis may also result from instrumentation during antenatal medical procedures.

Cultures of fluid taken perinatally are often mixed and include streptococci, anaerobes, Enterobacteriaceae, *"Streptococcus anginosus"* group, *Listeria monocytogenes* and *Mycoplasma hominis.* Other organisms that have been implicated in amniotic infections include enterococci, *Haemophilus* species, *Candida* species, aerobic Gram

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positive bacilli, pseudomonads and staphylococci. Proteomics may offer the best diagnostic option for this condition in the future[3].

Pericarditis[4]

Inflammation of the pericardium, the membrane enveloping the heart, is known as pericarditis. This results in an increase in the volume of fluid in this sac. However, most pericardial effusions are small in volume and are sterile.

Infectious pericarditis can be separated into three groups:

1. purulent, which are caused by bacteria and is fatal if untreated. It has a 40% mortality in patients who are treated. A wide range of bacteria have been isolated from cases of purulent pericarditis
2. benign, either due to viruses or post pericardiotomy syndrome
3. hypersensitivity or post-infectious

In AIDS pericarditis, the incidence of bacterial infection is much higher than in the general population, with a higher rate of *Mycobacterium* species infections[5].

Peritonitis

Peritonitis is inflammation of the peritoneum, the serous membrane lining the abdominal cavity and covering the abdominal viscera. Primary bacterial peritonitis accounts for <1% of bacterial peritonitis and occurs spontaneously without evidence of intra-abdominal organ perforation. It is most frequently seen in children and particularly those with nephrotic syndrome.

Spontaneous bacterial peritonitis (SBP) is the infection of pre-existing ascites in the absence of known intra-abdominal infection, and is a frequent, serious complication of cirrhosis and other liver disease. Infection is almost always mono-microbial, usually resulting from haematogenous spread. Lactoferrin levels can prove a useful way to identify this infection[6-8].

Secondary bacterial peritonitis usually arises following gastrointestinal leakage within the peritoneal cavity. This leakage may follow perforation of diseased viscera or abdominal trauma. The commonest cause in western countries is acute appendicitis. Other causes include perforated peptic ulcer, diverticular disease of the colon, pancreatitis and cholecystitis and as a complication of CAPD (see B 25 – Investigation of continuous ambulatory peritoneal dialysis fluid).

Localised peritonitis develops over any inflamed area of the gastrointestinal tract. It is a milder condition that may resolve, but may leave residual adhesions.

Acute generalised peritonitis is an extremely serious and often fatal condition. It usually arises as a consequence of leakage of gastrointestinal tract contents from a perforated ulcer or from a ruptured gangrenous appendix. The large quantity of bacterial toxins absorbed often leads to the development of paralytic ileus, toxaemia and septic shock.

Chronic peritonitis may develop as a result of abscess formation and persist for weeks or months unless drained. Persistent abscesses can cause general ill health and may become surrounded by dense fibrous tissue which interferes with the function of the intestinal loops. Chronic infection may also be caused by *M. tuberculosis*.

Pleurisy

Pleurisy is inflammation of the pleura, the serous membranes that cover the lungs and the inner aspect of the thoracic cavity.

Pleural effusion

Pleural effusion is the accumulation of fluid between the inner and outer (visceral and parietal) layers of the pleura. It may arise as the result of pneumonia, chronic heart failure or uraemia (when cultures will be negative in the latter two), or by direct spread of infection, such as a primary tuberculous focus rupturing into the pleural cavity. Carcinomatous involvement of the visceral pleura is one of the more common causes of sterile pleural effusions.

Effusion occurs early in the course of pneumonia representing the pleural response to an inflammatory reaction in the adjacent lung[9]. Bacteria reach the pleural space by various routes: spreading from an adjacent area of pneumonia, thoracic surgery or drainage, bacteraemia, chest trauma or by trans-diaphragmatic spread from intra-abdominal infection.

Tuberculous pleural effusion usually arises as an extension of infection from a subpleural focus. Only small numbers of bacilli are found in the effusion, and as a result microscopy is rarely positive. Therefore other confirmatory tests are preferred eg sputum examination, skin tests or chest radiography[10].

Empyema

Empyema thoracis is collection of pus in the pleural cavity. It most often occurs as a complication of bacterial infection of the pulmonary parenchyma, either pneumonia or lung abscess.

Whereas the most common cause is *S. pneumoniae,* any organism can be isolated from pleural fluid, in particular organisms associated with lower respiratory tract infection and organisms acquired by aspiration of the oropharyngeal flora, including oral streptococci and anaerobes.

Organisms particularly associated with empyema in patients with acquired immune deficiency syndrome (AIDS) include: *Cryptococcus neoformans, Mycobacterium avium-intracellulare, M. tuberculosis* and *Nocardia asteroides*[11,12].

Other organisms which may cause infection in this group of patients include *Pneumocystis jirovecii* and *Rhodococcus equi*[13].

Septic arthritis[14,15]

Septic arthritis is a pyogenic infection of a joint. Infection occurs via haematogenous spread or directly from contiguous lesions. Signs of infection may be difficult to detect clinically in patients whose joints are already inflamed due to rheumatological conditions. Patients with longstanding rheumatoid arthritis and osteoarthritis are predisposed to septic arthritis. Other predisposing factors include a history of trauma or intra-articular injection, immunosuppression, diabetes mellitus and malignancy. The aetiology of sepsis in prosthetic joints differs from that of non-prosthetic joints.

Infected synovial fluid is usually turbid or purulent with >75% of cells being polymorphonuclear leucocytes, although this is not specific for septic arthritis.

Any organism may be isolated from joint fluid, the most frequent isolates being: *Staphylococcus aureus,* streptococci, Enterobacteriaceae, *M. tuberculosis, Neisseria*

gonorrhoeae. *S. pneumoniae* and *Kingella kingae* are common isolates from children[16]. As a result of immunisation, infection with *Haemophilus influenzae* type b is now less common.

Purulent arthritis and synovitis may also be caused by sodium urate crystals (gout) and calcium pyrophosphate crystals (pseudo-gout). If required, microscopic examination of synovial fluid can be performed under polarised light.

Bursitis[17]

Bursitis is the inflammation of a bursa; a small, fluid-filled sac of fibrous tissue lined with synovial membrane formed around joints and places where ligaments and tendons pass over bones. It is often accompanied with prominent overlying cellulitis. The olecranon and prepatellar bursae are the most commonly affected sites. They are often subjected to repeated trauma. Skin wounds are the most likely portals of entry of infection and *S. aureus* is the most common isolate.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[18,19]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

1 Safety considerations[18-34]

1.1 Specimen collection, transport and storage[18-23]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[18-34]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[26].

Where Hazard Group 3 organisms eg *Mycobacterium tuberculosis* are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

All specimens from the pleural cavity must be centrifuged in sealed buckets and processed in a microbiological safety cabinet under full containment level 3 conditions, whether or not examination for *Mycobacterium* species is requested.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

If blood culture bottles are employed to provide an enrichment broth then any consequential use and subsequent disposal of syringes and needles must comply with local safety protocols.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Amniotic fluid, bursa fluid, pericardial fluid, synovial (joint) fluid, peritoneal fluid (ascites), pleural fluid

2.2 Optimal time and method of collection[35]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[35].

Samples of fluid rather than swabs of the fluids are the preferred specimen type to facilitate comprehensive investigation.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens[35]

Ideally, a minimum volume of 1mL.

Large volume specimens such as peritoneal fluid and ascitic fluid may contain very low numbers of organisms which require concentration in order to increase the likelihood of successful culture.

Small volume fluids such as synovial fluids may be received in insufficient volumes. This may impede the recovery of organisms.

The number and frequency of specimens collected depend on the clinical condition of the patient.

3 Specimen transport and storage[18,19]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[35].

If acute infection is suspected and the result may affect medical management, receive and process the sample within 4 hours. The result for microscopy should be made available within 2hr of the Gram stain.

If processing is delayed, refrigeration is preferable to storage at ambient temperature.

4 Specimen processing/procedure[18,19]

4.1 Test selection

Divide specimen on receipt for appropriate procedures such as microscopy and culture for *Mycobacterium* (B 40 – Investigation of specimens for *Mycobacterium* species), and/or Legionella.

4.2 Appearance

Describe colour, opacity and if a clot is present.

4.3 Sample preparation

For all except clotted or very viscous specimens:

- centrifuge in a sterile, capped, conical-bottomed container at 1200xg for 5-10mins or use a cytospin preparation

  Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20mins and the same deposit used for this as well as routine microscopy and culture

- transfer all but the last 0.5mL of the supernatant using a sterile pipette to another CE Marked leak proof container in a sealed plastic bag, for additional testing if required (eg virology)

- resuspend the deposit in the remaining fluid

4.4 Microscopy (refer TP 39 – Staining procedures)

4.4.1 Standard

Gram stain

For all except clotted or very viscous specimens:

- place one drop of centrifuged deposit using a sterile pipette on to a clean microscope slide
- spread this with a sterile loop to make a thin smear for Gram staining

Clotted specimens

If possible, the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram staining.

Total white cell count

The presence of a clot will invalidate a cell count.

If specifically requested for the differential diagnosis of Spontaneous Bacterial Peritonitis, or according to local protocol, perform a total cell count on the uncentrifuged specimen in a counting chamber. A full blood count, as well as a differential count, can also be performed using automated blood-cell analysers provided that they have been validated for body fluid microscopies, on specimens other than blood, and provided that the specimens which are acceptable are defined along with exclusion criteria and in which circumstances a manual microscope might be preferable[36-39].

4.4.2 Supplementary

Differential leucocyte count

Differentiating between polymorphonuclear leucocytes and mononuclear leucocytes may be performed in two ways:

1. **Counting chamber method: recommended for lower WBC counts.**

   a) Non- or lightly-bloodstained specimens

- stain the uncentrifuged fluid with 0.1% stain solution such as toluidine, methylene or nile blue. This stains the leucocyte nuclei thus aiding differentiation of the cells
- the dilution factor must be considered when calculating the final cell count
- count and record the numbers of each leucocyte type
- express the leucocyte count as number of cells per litre

   b) Heavily bloodstained specimens

- dilute specimen with WBC diluting fluid and leave for 5 minutes before loading the counting chamber. This will lyse the red blood cells and stain the leucocyte nuclei for differentiation
- count and record the number of each leucocyte type. The dilution factor must be considered when calculating the final cell count
- express the leucocyte count as number of cells per litre

2. **Stained method**

   Recommended for very high WBC counts where differentiation in the counting chamber is difficult

- prepare a slide from the centrifuged deposit or cytospin preparations as for the Gram stain but allow to air dry
- fix in alcohol and stain with a stain suitable for WBC morphology

  Note: Heat fixation distorts cellular morphology

- count and record the number of each leucocyte type as a percentage of the total

Microscopy for crystals

Performed only on request or according to local protocols

- examine the centrifuged deposit for the presence of crystals with a polarising microscope (sometimes such examinations are referred to other departments or pathology disciplines such as rheumatology, histopathology or cytology) depending on local protocols
- the needle-shaped, birefringent crystals of sodium urate are diagnostic of gout
- the rod or rhomboid-shaped crystals of calcium pyrophosphate are weakly birefringent and are indicative of pseudo-gout. Note that joints affected by gout can be secondarily infected

Other microscopy

- microscopy for *Mycobacterium* species - see B 40 – Investigation of specimens for *Mycobacterium* species
- direct immunofluorescent antibody for *Legionella* species
- indirect immunofluorescent antibody test for *P. jirovecii* (often performed in other pathology disciplines, eg histology)

Note: Methods for staining procedures and immunofluorescent techniques are contained in separate SMIs.

4.5 Culture and investigation

Pre-treatment

Standard

Centrifuge specimen (already performed for microscopy – see Section 4.4).

Note: Every sample should be cultured regardless of cell count.

If blood culture bottles are used, inoculate bottles with the uncentrifuged specimen, ideally at the “bedside”.

Supplementary

Mycobacterium species - see B 40 – Investigation of specimens for *Mycobacterium* species.

Specimen processing

Inoculate each agar plate and the enrichment broth with the centrifuged deposit (see Q 5 – Inoculation of culture media for bacteriology) using a sterile pipette.

For the isolation of individual colonies, spread inoculum with a sterile loop.

Clotted specimens

Inoculate the clot fragments to the agar plates and the enrichment broth.

If the specimen contains only a small clot, this should be included either in the enrichment culture or inoculated onto the chocolate agar plate. The unclotted portion of the specimen should be cultured in the normal way as described above.

Supplementary

If culture negative from a patient where infection is strongly implicated, consider other non-culture methods for identification eg 16S rDNA PCR, etc.

4.5.1 Culture media, conditions and organisms[1,40-43]

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Infection suspected in a normally sterile site	Amniotic fluid, bursa fluid, pericardial fluid, synovial (joint) fluid, peritoneal fluid (ascites), pleural fluid	Blood agar	35-37	5-10% CO_2	40-48hr*	daily	Any organism
		Fastidious anaerobe agar	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
		Chocolate agar	35-37	5-10% CO_2	40-48hr*	daily	Any organism
		If supplemented blood culture bottles† are used then that may replace the need for the plates outlined above, based on local risk assessment. Or anaerobic broth then subcultured to the plates above.	35-37	air	continuous monitoring	N/A	Any organism

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Peritonitis	Ascitic fluid Peritoneal fluid	Neomycin fastidious anaerobe agar	35-37	anaerobic	40 – 48hr*	≥48hr	Anaerobes
		CLED or MacConkey agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae

Clinical details/ conditions	Specimen	Optional media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
If microscopy suggestive of mixed infection	As appropriate	Staph/strep selective agar	35-37	air	16-24hr	≥16hr	*S. aureus* β-haemolytic streptococci
If fungi suspected clinically	As appropriate	Sabouraud or mycosel agar	35-37	air	21 days	10 and 21 days	Moulds and Yeasts

Other organisms for consideration –*Mycobacterium* (B 40), *Chlamydia* species, *Pneumocystis jirovecii,* viruses.

* plates can be incubated up to 5-7 days if required for example if *Nocardia* or *Actinomyces* is suspected.

† follow manufacturer's recommendations

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
All other organisms (including moulds and yeast)	species level
Mycobacterium species	B 40 - Investigation of specimens for *Mycobacterium* species

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Gram stain

Report on WBCs and organisms detected.

Cell count (if requested)

Report numbers of WBCs x 10^6 per litre.

Also report PMNs and mononuclear leucocytes as percentage of the total WBCs, if requested.

***P. jirovecii* immunofluorescence**

Report *P. jirovecii* cysts detected or not detected by immunofluorescence.

Microscopy for *Legionella* and *Mycobacterium* species (B 40 – Investigation of specimens for *Mycobacterium* species).

Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically within 2 hours of processing.

Written report, 16-72hr.

5.2 Culture

Report the organisms isolated or

Report absence of growth.

Also, report results of supplementary investigations.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[44,45] or equivalent in the devolved administrations[46-49]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

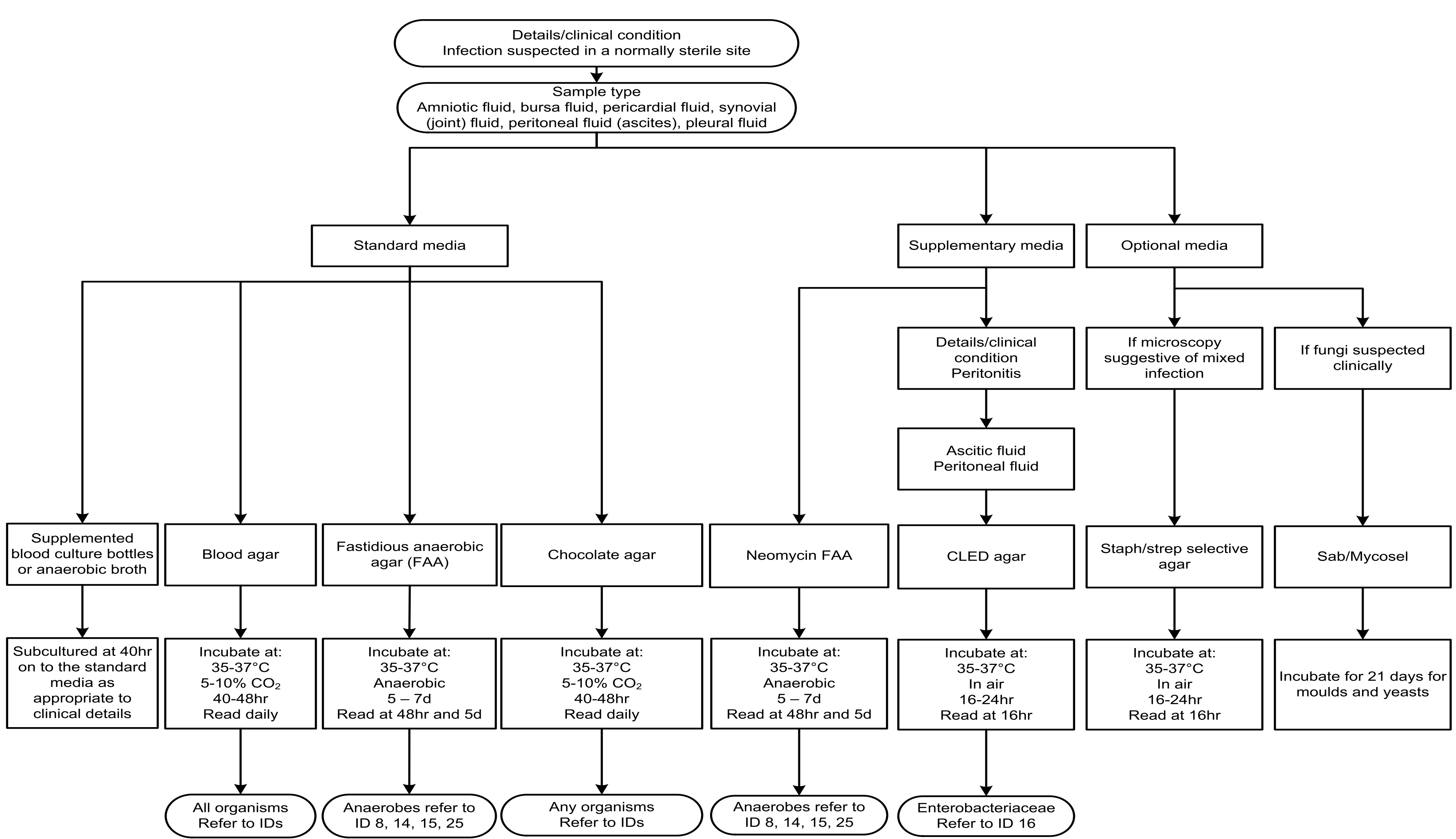
Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under

'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[46,47], Wales[48] and Northern Ireland[49].

Appendix: Investigation of fluids from normally sterile sites



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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 26 Investigation of Fluids from Normally Sterile Sites

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation: 28/01/2013 – 22/04/2013

Version of document consulted on: B 26dc+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	29/01/2013	**Lab Name**	NUH Nottingham
Section	2.5.2/ 2.5.3 and 2.5.3 table		
Comment			
Unclear if broth enrichment is routinely advised or an optional extra.			
Recommended Action	**NONE** Blood culture bottles are recommended as a form of enrichment. If these are used then plates do not need to be put up on the sample unless the bottle flags positive.		

Comment Number	2		
Date Received	31/01/2013	**Lab Name**	Hereford
Section	Introduction p9, p11 and table 2.5.3		
Comment			
The introduction suggests *H. influenza* is a target organism and few organisms may be expected yet the use of a 'supplemented' BHI broth is optional. a. Is the BHI supplement you suggest NAD? If so do you know of a commercial supplier for this product and do you recommend this? b. Is it your suggestion that *H. influenza* is unlikely to be isolated and so the decision to use ie BHI+ NAD or blood culture bottles is a local decision.			
Financial Barriers			
Lack of a commercial source of ready prepared BHI+			
Recommended Action	**ACCEPT** Removed from the UK SMI.		

Comment Number	3		
Date Received	31/01/2013	**Lab Name**	RIE
Section	Scope 2.1 2.5.3		
Comment			
a. Scope pouch of douglas fluid is excluded would it not be easier to include it as I cannot find it in the SOPs referenced.			

b. 2.1 Where Hazard Group 3 organisms e.g. *Mycobacterium tuberculosis* are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions. Our local risk assesments allow us to process samples at containment level 2 with use of additional controls such as class 1 safety cabinets if we are not attempting to culture *Mycobacterium tuberculosis* from the sample. All TB culture work is done at containment level 3. HSE are aware of this practice. c. 2.5.3 Media for actinomyces culture are not mentioned as an option- may be appropriate for pelvic samples and for other samples if clinically indicated. Prolonged culture for Nocardia may be clinically indicated in some samples. d. 16S PCR may be useful in some samples if culture negative.

Recommended Action	a. **NONE** This is covered in the under review B 28 and a cross reference to this document has been inserted. b. **NONE** c. **ACCEPT** UK SMI amended. d. **ACCEPT** UK SMI amended.

2nd Consultation: 27/09/2013 – 20/12/2013

Version of document consulted on: B 26di+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	30/09/2013	**Lab Name**	Nottingham Clinical Microbiology Dept
Section	2.5.5 Culture Table		
Comment			
In standard media section enrichment broths were optional in previous method and stated BHI or Blood culture bottles. You have now moved them into required section and changed to anaerobic broth, what is the data for this change. We have recently reviewed broth enrichment in these samples and have found it to be of limited benefit.			
Evidence			
Look back over workload for 12 months: Joint fluids (1249 samples) - 1.6% broth positive (1/4 of these significant) With the exception of Ascitic samples (566 samples) - 8.5% broth only positive (better return now use Blood culture bottles) all other sample types have poor return with enrichment broth.			
Chocolate agar and enrichment broth are now in the standard media section, do you have any comments on this?			

See above.			
Do you use enrichment broth?			
Yes.			
Health Benefits			
No.			
Recommended Action	**NONE** The option to use either enrichment or plates is given in the document.		

Comment Number	2		
Date Received	06/12/2013	**Lab Name**	Public Health Wales Rhyl
Section	2.4.2 supplementary		
Comment			
Great document. Just a couple of small points. a. The SOP refers to the counting chamber WBC differential method using Toluidene and WBC dilution fluid. For someone using this method for the first time there may not be enough detail, and the method is not available in TP 39 Staining procedures. b. The flow chart shows FAA Neomycin in the standard set. Shouldn't it be a supplementary media.			
Chocolate agar and enrichment broth are now in the standard media section, do you have any comments on this?			
Rhyl currently uses Supplemented Blood agar in place of Blood agar and Chocolate agar. This has been evaluated and found to be comparable, thus freeing up space, reducing waste and cost.			
Do you use enrichment broth?			
Yes.			
Which specimens do you culture in the context of sterile sites?			
As listed within the SMI.			
Do you have any views on the new presentation of the flowcharts?			
Well structured and easy to follow.			
Financial Barriers			
Financial barriers always present.			
Health Benefits			

Yes.	
Recommended Action	a. **ACCEPT** This will be included in the TP 39 document which is currently under review. b. **ACCEPT** UK SMI has been amended.

Comment Number	3		
Date Received	23/12/2013	**Lab Name**	Royal College of Physicians
Section	1.2 Achieving Optimal Conditions 1.2.1 Time between specimen collection and processing		
Comment			
Collect specimens before antimicrobial therapy where possible. Specimens should be transported and processed as soon as possible. Our experts believe that the guidance should be more specific than 'as soon as possible'. For example, if a clinical specimen is obtained during the night, should a lab scientist be called in immediately to process it or should it wait until the following morning?			
Recommended Action	**ACCEPT** Reference has been added to these sections to give more information on this area.		

Comment Number	4		
Date Received	20/12/2013	**Lab Name**	Mycology Reference Laboratory
Section	Many		
Comment			
There are not many comments from a mycology point of view but one of our members did a full job on the documents and I thought it might be useful so I have attached the amended document for your consideration.			
Recommended Action	**ACCEPT** The document has been amended.		

COMMENTS RECEIVED OUTSIDE OF CONSULTATIONS

Comment Number	1		
Date Received	07/01/2013	**Lab Name**	MSTAG
Section	a. Pericarditis b. Centrifugation c. Vortexing d. Differential leucocyte counts e. 2.5.3 f. 2.7		
Comment			
a. Some typos in some peoples versions-not seen on word version. b. Not always appropriate-ie very viscous Synovial fluids. c. Although it affects the “air-curtain”, it is just as risky to vortex outside-ie what happens if tube breaks. Also the term “curtain” is this a reference to a Type 2 cabinet as Type 1s do not have an air curtain. d. Many labs use cytospin preparations or centrifuged deposits to perform a differential count, which is different from the total WBC which would be performed on uncentifuged sample. e. i. If chocolate agar was added to this table it would simplify the algorithm. ii. Discussion on enrichment broths-what is supplemented vs. non supplemented. iii. Inconsistent with prosthetics NSM. iv. CLED/Mac-16h-why not 18-24h. f. Should BSAC be only one mentioned here as many labs use more than one method or different method.			
Recommended Action	a. **ACCEPT** The spelling in the document has been checked. b. **ACCEPT** It is not always possible to centrifuge the sample. c. **NONE** Sentence not present in the document. d. **ACCEPT** This option has been added in to the document. e. i. **ACCEPT**		

	UK SMI has been amended. ii. **ACCEPT** UK SMI has been amended. iii. **NONE** Different sample types. iv. **NONE** Standard time frame for this plate type in all our documents. f. **NONE** The group has agreed to continue to recommend BSAC until such a time as they become EUCAST.

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 12			
Date Received	29/01/2013	**Lab Name**	SRM Institute for Medical Services, Chennai, India
Date Received	29/01/2013	**Lab Name**	Guildford Nuffield Pathology
Date Received	31/01/2013	**Lab Name**	Microbiology, Glasgow
Date Received	13/02/2013	**Lab Name**	Golden Jubilee National Hospital
Date Received	15/03/2013	**Lab Name**	Microbiology, Newcastle Hospitals NHS Foundation Trust
Date Received	05/04/2013	**Lab Name**	Bristol
Date Received	16/04/2013	**Lab Name**	Sunderland Royal Hospital
Date Received	10/12/2013	**Lab Name**	Microbiology Dept, CPL,St James Hosp, Dublin 8, Ireland
Date Received	17/12/2013	**Lab Name**	Clinical Evidence & Effectiveness
Date Received	15/05/2014	**Lab Name**	Nottingham NUH
Date Received	30/05/2014	**Lab Name**	Truro, Cornwall

Date Received	31/05/2014	Lab Name	Truro, Cornwall

UK Standards for Microbiology Investigations

Investigation of Cerebrospinal Fluid

NICE accredited
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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 27 | Issue no: 6.1 | Issue date: 31.05.17 | Page: 1 of 29

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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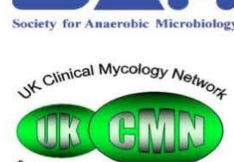

Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS ... 2

AMENDMENT TABLE ... 4

UK SMI: SCOPE AND PURPOSE ... 5

SCOPE OF DOCUMENT ... 7

SCOPE ... 7

INTRODUCTION ... 7

TECHNICAL INFORMATION/LIMITATIONS ... 12

1 SAFETY CONSIDERATIONS ... 13

2 SPECIMEN COLLECTION ... 14

3 SPECIMEN TRANSPORT AND STORAGE ... 14

4 SPECIMEN PROCESSING/PROCEDURE ... 15

5 REPORTING PROCEDURE ... 21

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS ... 22

APPENDIX: INVESTIGATION OF CEREBROSPINAL FLUID ... 24

REFERENCES ... 25

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	10/31.05.17
Issue no. discarded.	6
Insert Issue no.	6.1
Section(s) involved	**Amendment**
Diagnosis of meningitis.	Table referring to normal values of CSF has been updated to include a wider population: neonates, infants and elderly.

Amendment No/Date.	9/24.02.15
Issue no. discarded.	5.2
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope.	Cross reference to G 4 inserted.
Introduction.	Restructured so that organisms causing meningitis are at the beginning followed by clinical presentations. Normal CSF values table amended to include the ages and the supporting text underneath has been strengthened.
2.3 Adequate quantity and appropriate number of specimens.	Section clarified to describe how many and what kind of samples should be taken.
4.3.1 Culture Media.	Slopes added for long term culture of fungi. Culture recommendations for anaerobes have been strengthened.
4.5.2 Specimen Processing.	The use of 16S PCR and MALDI TOF inserted.

5.1 Microscopy reporting time.	Guidelines for reporting of cell counts have been given.
References.	References reviewed and updated.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested Citation for this Document

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Scope of Document

Type of Specimen

Cerebrospinal fluid

Scope

This SMI describes the examination of cerebrospinal fluid (CSF) for the detection and recovery of the causative bacterial or fungal organisms of meningitis. Viruses and other causes of meningitis are mentioned only briefly. For more information on viral meningitis refer to G 4 – Investigation of Viral Encephalitis and Meningitis and V 43 – Investigation of Viral Encephalitis.

This SMI should be used in conjunction with other SMIs.

Introduction

Meningitis is defined as inflammation of the meninges. This process may be acute or chronic and infective or non-infective. Many infective agents have been shown to cause meningitis, including viruses, bacteria, fungi and parasites.

Organisms Causing Meningitis

Species isolated tend to be characteristically, but not exclusively, associated with the age or predisposing status of the patient[1,2].

From neonates and babies up to 2 months of age: Lancefield group B streptococci, *Escherichia coli, Listeria monocytogenes*, herpes simplex virus and *Neisseria meningitidis*. Premature neonates requiring intensive care are at risk of *Candida* species meningitis as a result of candidaemia.

From children older than two months to young adults: *N. meningitidis, Streptococcus pneumoniae,* viruses (in particular enteroviruses) and *Haemophilus influenzae* type b. The incidence of *H. influenzae* type b meningitis in the UK has been greatly reduced by routine Hib immunisation[2].

From adults: *S. pneumoniae, N. meningitidis,* viruses and occasionally non-group b *H. influenzae.* Patients older than 60 years without other predisposing factors may develop *Listeria monocytogenes* infection.

Fungi such as *Histoplasma capsulatum, Cryptococcus* species and *Coccidioides immitis* may infect the meninges in disseminated infection[3].

Spirochetes such as *Treponema pallidum*, *Borrelia* and *Leptospira* species may cause meningitis as part of a generalised infection.

Parasites (such as the amoebae *Acanthamoeba* species and *Naegleria species*) occasionally cause meningitis. *Naegleria fowlerii* invades the meninges via the cribriform plate in freshwater swimmers who inhale small quantities of water, giving rise to florid meningoencephalitis with a high fatality rate.

The nematode *Angiostrongylus cantonensis*, which has a distribution mainly in South East Asia and has also reported from the Dominican Republic, may cause eosinophilic meningitis in infected persons[4].

Many other organisms have been documented to cause meningitis and cannot all be covered in this document.

Acute Bacterial Meningitis[2]

Acute bacterial meningitis is a medical emergency. Symptoms and signs of meningitis may evolve over a few days or have a rapid onset and fulminant course over a few hours. The clinical picture may be dominated by accompanying septicaemia, as with meningococcal infection. Untreated, mortality is high. It is imperative that any specimen taken from a patient is processed as rapidly as possible, to optimise clinical management. Typically, the CSF becomes infiltrated with neutrophil leucocytes and has raised protein and reduced glucose concentrations.

A number of conditions predispose individual patients to develop meningitis[1]. Abnormal post-surgical and traumatic communications between the subarachnoid space and colonised sites (eg the nose and paranasal sinuses following basilar skull fracture), presence of CSF shunts, presence of cochlear implants, meningomyelocoele and other congenital malformations, infections of contiguous sites (eg the middle ear cavity or paranasal sinuses) and tumours in close proximity to the central nervous system are some examples. As well as direct spread, meningeal infection may occur as a result of blood-borne seeding from a distant site. Patients with immune dysfunction (such as complement deficiency syndromes, or hypogammaglobulinaemia) or who are receiving immunosuppressive treatment are at increased risk of meningitis.

Mixed infections are rare but can occur with certain predisposing conditions[5,6]. They are associated with trauma, tumours or infections such as acute paranasal sinusitis that may extend directly to the meninges. Mixed infections may also arise by direct entry of organisms via fistulae or as a result of a ruptured brain abscess.

Viral Meningitis[7]

Viral meningitis is usually benign and complications are rare. The course is often subacute, evolving over two or three days. The major cause is enteroviral infection, especially in the summer and autumn months. Lymphocyte predominance in the CSF is typical but it must be remembered that early in the course of the disease, both neutrophils and lymphocytes (sometimes with neutrophil predominance) may be seen. CSF glucose concentration is usually normal and protein concentration normal or slightly raised[1]. For more information refer to G 4 – Investigation of Viral Encephalitis and Meningitis.

Chronic Meningitis[8]

Chronic meningitis is said to be present when signs and symptoms of meningeal inflammation (including abnormalities in the CSF) have been present for a month or more.

A principal infective cause of this condition is tuberculous meningitis. In an established case the CSF may be infiltrated with lymphocytic cells. Tuberculous meningitis has insidious and protean clinical manifestations. It is generally rare in the UK but the diagnosis should be considered in patients from areas of high TB prevalence and in high risk groups. For further information see B 40 - Investigation of Specimens for *Mycobacterium* species.

Other Types of Meningitis[8]

Sarcoid meningitis is very rare and produces a raised protein concentration and leucocyte count together with lesions on the meninges seen on magnetic resonance imaging. Sarcoidosis is a multi-organ disease where the cause is unknown, although it has been postulated that it may be a result of the exposure of genetically susceptible individuals to infectious agents.

Carcinogenous meningitis arises from metastasis from a primary site to the meninges and diagnosis usually rests on the presence of cranial nerve lesion symptoms eg deafness, and by use of magnetic resonance imaging and cytological examination of the CSF for signet cells. It is also important to distinguish between true infection and the result of the malignancy because the two may co-exist.

Special Risk Groups

Patients who are immunosuppressed are additionally susceptible to meningitis caused by organisms such as *Listeria monocytogenes*, *Cryptococcus neoformans, Norcardia* and *Toxoplasma gondii*[9].

Patients with intracranial prosthetic material such as CSF shunts (see B 22 - Investigation of Cerebrospinal Fluid Shunts) are susceptible to infection caused by *Staphylococcus aureus*, coagulase-negative staphylococci, *Corynebacterium* species, *Propionibacterium* species, *Candida* species and *Enterobacteriaceae*.

Diagnosis of Meningitis[1,2]

Diagnosis of meningitis is best established by laboratory examination of the CSF. This is usually obtained by lumbar puncture, although ventricular, cisternal or fontanelle taps may also be used. Lumbar puncture may cause cerebral herniation, therefore in patients where there is a risk of increased intracranial pressure CT scanning is advised prior to the procedure. In some cases the patient is too unstable or has a bleeding diathesis as a result of sepsis syndrome and cannot undergo immediate lumbar puncture. Blood cultures and pharyngeal swabs may be useful in addition to CSF examination in the diagnosis of meningococcal meningitis and serology may allow retrospective diagnosis on acute and convalescent sera.

In patients for whom lumbar puncture is contraindicated, every effort must be made to establish a microbiological diagnosis by other means. This is desirable both for epidemiological purposes and for the appropriate management of contacts of cases.

The diagnosis of meningitis from the examination of CSF includes the following[1]:

- Complete cell count
- Differential leucocyte count
- Examination of Gram stained smear
- Culture
- Determination of glucose and protein concentrations (usually performed by clinical biochemistry departments)
- PCR where appropriate
- Antigen testing

Therapy should not be delayed pending CSF microscopy or culture. It is important to initiate effective antimicrobial therapy quickly, and this may commence before the examination of the CSF. Further early management decisions therefore, should be based on the immediate examination of the sample by cell count and Gram stain. Examination of the deposit by cytocentrifugation (eg Cytospin) is the most accurate method of cell differentiation but may not be routinely available.

PCR tests are available as a diagnostic procedure for viruses (for more information refer to G 4 – Investigation of Viral Encephalitis and Meningitis) and some other microorganisms although these techniques remain expensive and show differences in sensitivity and specificity between primer sets and laboratory set ups[2,10,11]. A broad-range bacterial PCR primer set has been established and this detects organisms that are found less frequently or that are unknown causative agents for bacterial meningitis[12]. It may be particularly useful in situations where culture is negative because of chemotherapy, and serology may also be helpful retrospectively in patients who survive. However accuracy of the 16S rDNA PCR approach differs depending on the sample, the microorganisms involved, the expected bacterial load and the presence of bacterial DNA other than that from the pathogen implied in the infectious disease[13].

The bacteria commonly causing meningitis carry specific polysaccharide surface antigens that can be detected by Latex Agglutination Test (LAT). LATs are expensive, reliability is disputed and sensitivity is poor[14]. LAT should not be used on CSF unless the cell count is abnormal, Gram stained film is negative and CSF and blood cultures remain negative after 48hr[14]. The clinician should be informed that, although a positive LAT indicates the presence of an infectious agent, a negative result is not definitive. The routine use of LAT is not recommended in this SMI.

CSF cryptococcal antigen testing should be carried out in all cases of suspected cryptococcal meningitis, and all cases of meningitis in immunocompromised patients in which there is an elevated CSF white cell count and no alternative diagnosis has been made[15]. In these cases serum should also be tested for cryptococcal antigen (CRAG).

Normal CSF values[16-25]

Leucocytes	Neonates	less 28 days	0-30 cells x 10^6/L
	Infants	1 to 12 months	0-15 cells x 10^6/L
	Children/Adults	1 year +	0-5 cells x 10^6/L
Erythrocytes	No RBCs should be present in normal CSF		
Glucose	Neonates	less 28 days	1.94-5.55 mmol/L
	Infants	29 to 58 days	1.55-5.55 mmol/L
		2-12 months	1.94-5.0 mmol/L
	Children/Adults	1 year +	2.22-4.44 mmol/L
Proteins	Neonates	less 28 days	0.65-1.5 g/L
	Infants	29-56 days	0.5-0.9 g/L
	Children	2 months to 18 years	0.05- 0.35 g/L
	Adults	over 60	0.15-0.6 g/L
		18 to 60	0.15-0.45 g/L

These values represent the approximate upper and lower limits of normality and are for guidance only.

Abnormalities associated with bacterial meningitis[1]

- Reduced glucose concentration: <60% blood glucose (CSF: serum ratio <0.6)
- Elevated protein concentration
- Raised white blood cell (WBC) count: 10^1 - 10^4 predominantly polymorphs
- Elevated intracranial pressure

The presence of RBCs in CSF can result from an intra-cerebral or sub-arachnoid haemorrhage or from a traumatic lumbar puncture (LP) in which peripheral blood contaminates the CSF. The presence of this contaminating blood may make interpretation of the CSF analysis more difficult but rarely obscures CSF abnormalities associated with bacterial meningitis[26].

Sequential samples 1 and 3, from one lumbar puncture, are examined. Uniform bloodstaining of all samples suggests previous haemorrhage into the sub-arachnoid space, whereas reducing counts in sequentially obtained samples suggest bleeding induced by the tap procedure.

A WBC:RBC ratio of 1:500 to 1:1000 is generally regarded as not indicative of infection. CSF obtained more than 12hr post intra-cranial haemorrhage may show raised WBC counts of up to 500 x 10^6/L as a result of an inflammatory response.

Although patients with untreated acute bacterial meningitis usually have high CSF polymorph counts, the CSF polymorph: lymphocyte ratio is unreliable as a pointer to the cause of meningitis. This is particularly so in neonates or when total leucocyte counts are less than 1000 x 10^6/L[2]. Viral meningitis is classically described as being associated with a lymphocytic CSF but neutrophils may predominate, especially early in the illness[27,28]. Tuberculosis meningitis may also be associated with a neutrophil rather than a lymphocytic infiltrate early in the infection[8]. Neutropenic patients may not produce reliable or characteristic polymorph or neutrophil responses in the CSF.

Occasionally examination of a wet preparation or performance of an India ink preparation will be indicated for the detection of amoebae and *C. neoformans* respectively. The latter is essential if cryptococcal infection is suspected in a patient who is immunocompromised, this should be confirmed by latex agglutination[14].

Xanthochromia

Xanthochromia is yellow colouration of the supernatant of centrifuged CSF. It can result from the metabolism of products of RBC breakdown, increased CSF protein concentration, or bilirubin staining. RBC breakdown in CSF commences approximately 1-2 hours post haemorrhage. The supernatant may initially be pink in colour due to the presence of oxyhaemoglobin. After 24 hours, the supernatant begins to show increasing xanthochromia caused by the degradation of oxyhaemoglobin to bilirubin. This usually peaks at 36-48 hours.

In sub-arachnoid haemorrhage xanthochromia is associated with a ten-fold increase in protein to ≥1.5g/L which peaks at 8-10 days post onset and then declines. In a fresh, traumatic lumbar puncture the CSF supernatant is usually clear and colourless, although other factors may contribute to its appearance[26].

Visual determination is unreliable. Xanthochromia should be determined by examination of the supernatant of centrifuged CSF by spectrophotometry to seek macroscopically invisible haematin or bilirubin, which, if present, will confirm pre-tap intracranial haemorrhage[29].

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[30,31]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

1 Safety Considerations[30-46]

1.1 Specimen Collection, Transport and Storage[30-35]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[30-46]

The processing of most diagnostic work can be carried out at Containment Level 2 unless infection with a) *N. meningitidis,* b) a Hazard group 3 organism or c) TSE is suspected.

a) *N. meningitidis* causes severe and sometimes fatal disease. Laboratory acquired infections have been reported. The organism infects primarily by the respiratory route. An effective vaccine is available for some meningococcal groups.

N. meningitidis is a Hazard group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2.

Due to the severity of the disease and the risks associated with generating aerosols of the organism, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols).

b) Where Hazard Group 3 *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

c) Refer to https://www.gov.uk/government/groups/advisory-committee-on-dangerous-pathogens for guidance on TSE agents. Laboratory policies that take into account the local risk assessments may dictate that the use of a microbiological safety cabinet should be used when dispensing the specimen.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[38].

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65-75°C), under the hood, until dry. Then place in a rack or other suitable holder.

Note: Heat-fixing may not kill all *Mycobacterium* species[47]. Slides should be handled carefully.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

CSF

2.2 Optimal Time and Method of Collection[48]

For safety considerations refer to Section 1.1.

Collect specimens preferably before antimicrobial therapy is started, but this must not be delayed unnecessarily pending lumbar puncture and CSF culture[48].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Specialist collection according to local protocols.

2.3 Adequate Quantity and Appropriate Number of Specimens[48]

CSF is normally collected sequentially into three or more separate containers which should be numbered consecutively. Collect specimens in appropriate CE marked leak proof containers and transport specimens in sealed plastic bags.

Collection of an additional sample in a container with fluoride for glucose estimation is also recommended, although such tubes should be filled last because they may contain environmental bacteria which might otherwise contaminate samples for culture.

Common practice is to send the first and last specimens taken for microbiological examination and the second specimen for protein. The fluoride sample should not be sent to Microbiology. Ideally testing should be carried out on the last sample with the first one reserved as a backup.

Ideally a minimum volume of 1mL for each tube 1 and 3 taken for microscopy (in adults). When sample volume is below this it is possible to pool samples.

For *Mycobacterium* species*,* at least 10mL where possible.

Note: The larger the volume, the greater the cultural yield particularly in relation to *M. tuberculosis* investigations.

3 Specimen Transport and Storage[30,31]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Time between collection to microscopy and culture should occur within a maximum of 2 hours[1,49]. Cells disintegrate and a delay may produce a cell count that does not reflect the clinical situation of the patient.

Specimens should be transported and processed as soon as possible[48].

Do not refrigerate specimen until after microscopy and bacterial culture have been performed. The specimen should then be refrigerated pending further investigation.

4 Specimen Processing/Procedure[30,31]

4.1 Test Selection

Specimens taken after routine neurological examination (eg myelogram, multiple sclerosis) do not require Gram film or culture unless the leucocyte count is raised, or these tests are clinically indicated or specified in local protocols.

Divide specimen, if multiple samples are not taken after performing microscopy and bacterial culture, for appropriate procedures such as protein estimation, culture for *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species), examination for parasites (B 31 - Investigation of Specimens other than Blood for Parasites), screening for cryptococcal antigen or virology as may be appropriate in view of clinical details, tests requested or microscopy results.

Note: If there is an insufficient volume of sample for all investigations, these should be prioritised following medical microbiological advice.

Rapid screening for antigens in CSF from cases of bacterial meningitis is not recommended routinely. However, it may be useful for example when deciding if two or more cases of the same type have occurred in a school (to guide mass prophylaxis or vaccination).

PCR is available as a diagnostic procedure for some organisms. An unopened sample, if available, is preferred for PCR.

4.2 Appearance

Describe turbidity and whether a clot is present (which would invalidate the cell count).

In extreme cases of TB meningitis a typical 'spider-web' clot may be present. Although rarely seen, its presence should be noted.

Record if the estimated specimen volume is insufficient for all investigations to be performed and obtain medical microbiological advice about prioritisation if appropriate.

Describe colour of supernatant after centrifugation.

Confirmation of xanthochromia should be performed by spectrophotometry if requested or if clarification of the source of RBCs in the CSF is required[29]. This is often carried out by clinical biochemistry departments as are protein and glucose determinations.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

See Microscopy section.

4.4 Microscopy

4.4.1 Standard total cell count

Perform total WBC and RBC counts on the uncentrifuged specimen, preferably the last specimen taken, using a counting chamber.

Cell counts should not be performed on specimens containing a clot (which invalidates the result).

Differential leucocyte count

1. **Counting chamber method (recommended for lower WBC counts)**

   a) **Non- or lightly bloodstained specimens**

Stain the unspun CSF with 0.1% stain solution such as toluidine, methylene or Nile blue. These stain the leucocyte nuclei aiding differentiation of the cells. If the CSF is diluted when adding the stain, remember to take the dilution factor into account when calculating the final cell count.

Count and record the actual numbers of each leucocyte type. Express the leucocyte count as number of cells per litre.

   b) **Heavily bloodstained specimens**

Dilute specimen with WBC diluting fluid and leave for 5 min before loading the counting chamber. This will lyse the RBCs and stain the leucocyte nuclei for differentiation.

Count and record the actual numbers of each leucocyte type. Taking the dilution factor into account, express as number of cells per litre.

2. **Stained method (recommended for very high WBC counts where differentiation in the counting chamber is difficult)**

Prepare a slide from the CSF centrifuged deposit as for the Gram stain, but allow to air dry. Fix in alcohol and stain with a stain suitable for WBC morphology.

Note 1: Heat fixation distorts cellular morphology.

Note 2: Count and record the actual numbers of each leucocyte type. Taking the dilution factor into account, express as number of cells per litre.

Note 3: A cytocentrifugation deposit (eg Cytospin) permits the most accurate cell differentiation. Care should be taken to use a sterile tube if this deposit is to be used for Gram stain examination.

Total red cell count

If haemorrhage is suspected, perform a total RBC count on a minimum of two specimens from the same lumbar puncture to assess uniformity of bloodstaining. Isotonic or phosphate buffered saline should be used for any dilutions required.

Gram stain (refer to TP 39 - Staining Procedures)

Perform Gram stain on all specimens except:

- Clotted specimens (see below)
- Routine neurological specimens unless leucocyte counts are raised
- PM specimens cell counts are unreliable but should be cultured

Centrifuge in a sterile, capped, conical-bottomed container at 1200 xg for 5-10 min.

Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20 min at 3000 xg (see B 40 - Investigation of Specimens for *Mycobacterium* species) and the same deposit used for this as well as routine microscopy and culture[50].

Transfer all but the last 0.5mL of the supernatant with a sterile pipette to another sterile container for additional testing if required (eg protein, virology).

Resuspend the deposit in the remaining fluid.

Place one drop of centrifuged deposit with a sterile pipette on a clean microscope slide.

Spread this with a sterile loop to make a thin smear for Gram staining.

The sensitivity of the Gram stain may be improved by serial drops being "built up" on the slide after each drop has dried, to maximise the amount examined. Care should be taken to ensure that the smear does not wash off during staining.

Clotted specimens

If possible the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram staining.

4.4.2 Supplementary

Examination for *M. tuberculosis*

The "build up" technique for films as described above is recommended for the examination for *Mycobacterium* species (see B 40 - Investigation of Specimens for *Mycobacterium* species). If a 'spider-web' clot is present this should be included in the portion of the specimen examined by microscopy and culture.

Examination for *C. neoformans*

Mix a drop of the centrifuged deposit with a drop of 50% aqueous India ink or nigrosin on a clean microscope slide and cover with a coverslip (see TP 39 - Staining Procedures).

Examine for the presence of round or oval yeasts with a clear halo around the cell, indicating the presence of a capsule. The presence of a capsule permits a presumptive identification of *C. neoformans*.

Examination for amoebae

Examine both uncentrifuged and centrifuged deposits as wet preparations. Place a drop of specimen on a clean microscope slide, cover with a coverslip and examine for amoebic trophozoites (B 31 - Investigation of Specimens other than Blood for Parasites).

4.5 Culture and Investigation

4.5.1 Pre treatment

Standard

Centrifuge specimen (already performed for microscopy - see 4.4).

Supplementary

Mycobacterium species (B 40 - Investigation of Specimens for *Mycobacterium* species) and parasites (see B 31 - Investigation of Specimens other than Blood for Parasites).

4.5.2 Specimen processing

Standard

For all CSF

- With a sterile pipette inoculate each agar plate with the centrifuged deposit (see Q 5 - Inoculation of Culture Media for Bacteriology)
- Allow inoculum to dry before spreading to minimise any antibiotic effect which may be present
- Spread inoculum with a sterile loop for the isolation of individual colonies

Clotted specimens

Inoculate the clot fragments to each agar plate.

If the specimen contains only a small clot, this should be included in the inoculum applied to the chocolate agar plate. The unclotted portion of the CSF should be cultured in the normal way as described above.

Supplementary

If culture negative result from clinically ill patient consider other non-culture methods for diagnosis eg 16S PCR, MALDI TOF, etc.

Broth cultures are not recommended as a significant positive yield is rarely achieved and contamination is frequent, unless dealing with shunt infections where they may add value[51-53].

4.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Meningitis Post neurosurgery Reservoirs Ventriculitis Immunocompromised	CSF	Chocolate agar	35 - 37	5 - 10% CO_2	40-48hr	daily	Any organism
		Blood agar	35 - 37	5 - 10% CO_2	40-48hr	daily	

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Immunocompromised patients	CSF	Sabouraud plate	35 - 37	air	14d*	≥ 40hr:	Fungi
Brain abscess Ventriculitis Reservoirs Post neurosurgery Post otitis media with complications	CSF	Fastidious anaerobe agar	35-37	anaerobic	10d	≥40hr, 5d and at 10 days if you have an anaerobic cabinet otherwise at 10days.	Anaerobes
If mixed infection suggested by Gram stained film	CSF	Neomycin fastidious anaerobe agar	35-37	anaerobic	10d	≥40hr, 5d and at 10 days if you have an anaerobic cabinet otherwise at 10 days	

* If longer culture times are likely to be required a sabouraud slope should be put up in addition to the plate.

Enrichment broths may add value when diagnosing shunt infections (see page 18).

Other organisms for consideration - *Mycobacterium* species and parasites as described in supplementary testing, *T. pallidum* and viruses can be found in relevant SMIs.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	species level
Actinomyces	species level
β-haemolytic streptococci	Lancefield group level

All other organisms	species level
Mycobacterium	B 40 - Investigation of Specimens for *Mycobacterium* species
Parasites	B 31 - Investigation of Specimens other than Blood for Parasites

Note: Any organism considered to be a contaminant may not require identification to species level.

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

β-haemolytic streptococci	Serotyping
S. pneumoniae	Serotyping
H. influenzae	Serotyping
Listeria species	Serotyping
N. meningitidis	Strain characterisation, antimicrobial susceptibility testing
Fungi	Identification and/or susceptibility testing
Mycobacterium species	B 40 - Investigation of Specimens for *Mycobacterium* species

Isolates associated with outbreaks, where epidemiologically indicated and organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

CSF, EDTA blood and paired serum samples may be sent to the Meningococcal Reference Unit (MRU) for examination using molecular methods and serological examination if culture is negative and meningococcal infection suspected.

Specimens for molecular testing for other organisms may be sent to appropriate laboratories if clinically indicated.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Appearance

Report the appearance of the CSF and the presence of a clot if applicable.

5.2 Microscopy

Cell count

Report numbers of RBCs x 10^6 per litre and

Report numbers of PMNs and lymphocytes x 10^6/L or

Report PMNs and lymphocytes as percentages of the total WBC (which is reported as x 10^6).

In certain cases referral to cytology for identification of mononuclear and other cells may be indicated.

Gram stain

Report on organisms detected and presence or absence of pus cells.

Supplementary

India ink or nigrosin.

Report on encapsulated yeasts detected.

Microscopy for *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of Specimens other than Blood for Parasites).

5.2.1 Microscopy reporting time

Results of cell counts and stains should be communicated immediately, within two hours of receiving the specimen and made available on the clinical users' results viewing system. Where such facilities are not available, written or computer generated reports should follow preliminary/verbal reports within 24 hours.

5.3 Culture

Report the organisms isolated or

Report absence of growth.

Also, report results of supplementary investigations.

Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically when available.

Interim/final written report, 16–72 hours stating, if appropriate, that a further report will be issued.

Molecular testing results (if applicable).

Supplementary investigations: *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species) fungi (B 39 - Investigation of Dermatological Specimens for Superficial Mycoses) and parasites (B 31 - Investigation of Specimens other than Blood for Parasites).

5.4 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[54,55] or Equivalent in the Devolved Administrations[56-59]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[56,57], Wales[58] and Northern Ireland[59].

Clinically significant isolates from CSF should be reported to the Regional CIDSC and Local CCDC.

Refer to the following:

Individual SMIs on organism identification.

Health Protection Agency publications:

"Laboratory Reporting to the Health Protection Agency. Guide for diagnostic laboratories".

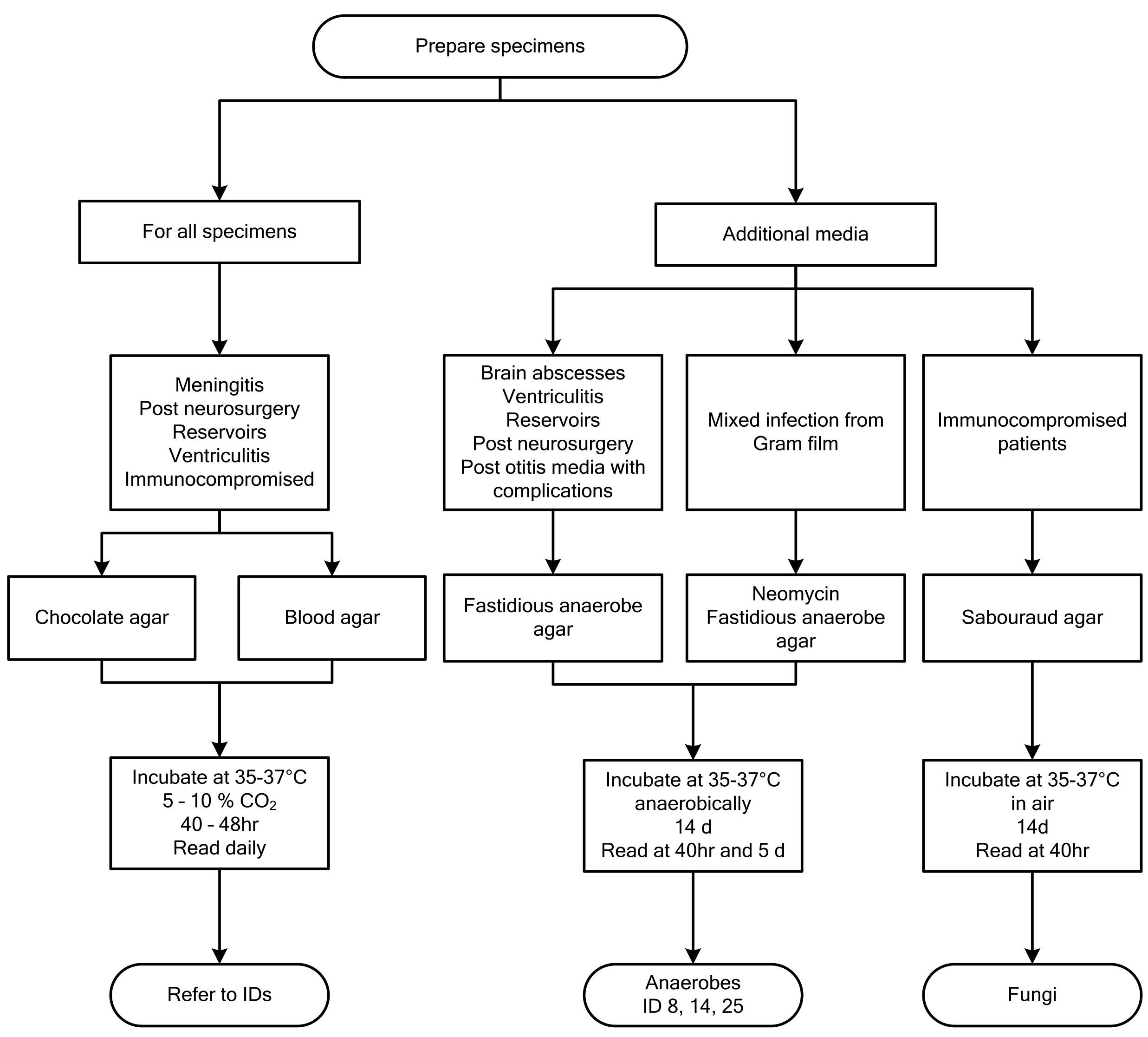
Local Memorandum of Understanding.

Current guidelines on CIDSC and COSURV reporting.

In cases of suspected meningococcal disease and contacts the isolation of *N. meningitidis* should be reported to the CCDC immediately.

Report all isolates of the following: *Mycobacterium* species.

Appendix: Investigation of Cerebrospinal Fluid



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UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 27 Investigation of Cerebrospinal Fluid

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 21/09/2012 – 14/12/2012

Version of document consulted on: B 27dc+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	11/12/2012	**Lab Name**	on behalf of the UK Clinical Mycology Network
Section	Various		
Comment			

a. The reference for normal CSF RBC and WBC values is a 20 year old review article, and the original article includes a 'caution' about the interpretation of these values (http://cmr.asm.org/content/5/2/130.full.pdf). It is rather simplistic to state that 0-30 is a 'normal' WBC value for a neonate, when, in fact, meningitis may be diagnosed in neonates with pretty much any CSF WBC value. Furthermore, it appears to be inaccurate, in the light of (for instance) the article at http://pediatrics.aappublications.org/content/125/2/257.long, which is a modern attempt to define CSF WBC values in infants. This whole table needs to be revised according to recent literature.

b. 2.5.3 recommends a sab plate is put up in the immunocompromised although this would miss cryptosporidium as a presenting feature of HIV and also cases in non-immunocompromised.

   A sab plate would miss cryptosporidium as a presenting feature of HIV.

c. Also it is to be read at 40hrs and kept for UP TO 8 WEEKS! A sab plate cannot usefully be kept up for this long. If you have evidence that cultures need to be so prolonged, then slopes would have to be kept.

d. Is it still considered correct that C. neoformans doesn't show up well on Gram stain (see 1980 ref. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC273699/)? If so this should be made clear (with a reference), otherwise there would be no point in doing an India ink test in the presence of a negative the Gram stain. Notwithstanding the above, does the India Ink test add anything to a combination of Gram stain + CRAG, especially now that there is a lateral flow immunochromatography test available for CRAG? The India ink test is probably not very sensitive (microscopy generally isn't) and may result in a false sense of security.

e. It is very important that the CRAG test is carried out on a supernatant rather than native CSF - although this may be obvious I'm not sure the document makes it clear.

f. There should be some attempt made in this document to propose how Gram stain, India ink and CRAG fit in with each other, rather than simply mentioning all three.

g. Cryptococcal antigen (CRAG) may also be detected by LAT, although testing of serum is more sensitive than testing CSF alone'. There are a number of different types of CRAG test available, and this document should not dwell on any particular method. Also, it sort of implies that testing serum is more sensitive than

testing CSF. I doubt if many laboratories in the UK are using latex agglutination and most will use ELISA or have moved to the lateral flow device. Also an SOP should give an indication of when to perform the test. We suggest 'CSF Cryptococcalantigen testing should be carried out in all cases of suspected cryptococcal meningitis, and all cases of meningitis in immunocompromised patients in which there is an elevated CSF white cell count and no alternative diagnosis has been made. In these cases serum should also be tested for CRAG. h. Re. B27:1. The paragraph that starts 'Leukaemic meningitis' is a bit strangely worded. If leukaemia is mentioned it would more appropriately be described as 'Meningitis is rare in association with leukaemia, but...' However, I suggest that this paragraph is redundant, and it is sufficient to refer to Cryptococcus neoformans in the general paragraph about immunosuppression.	
Recommended Action	a. **NONE** The valves listed in the table represent the approximate upper and lower limits of normality particularly in neonates and children. b. **NONE** Methods for the diagnosis cryptosporidium is covered in other parts of the document. c. **ACCEPT** Document amended to include Sabouraud slope if longer incubation time is required. d. **NONE** It is the opinion of the working group that India Ink remains as a test. e. **ACCEPT** The document has been amended. f. **NONE** This should be decided at a local level. g. **ACCEPT** Recommended text inserted in to the document. h. **ACCEPT** Paragraph removed.

Comment Number	2		
Date Received	10/12/2012	**Lab Name**	Department of Medical Microbiology, Conquest Hospital
Section	1.2.2		
Comment			
CSFs should be examined immediately. Evidence shows that to obtain accurate cell counts they should be examined within one hour of the sample being taken from a patient and certainly within two hours (by which time up to 50% of cells can lyse). The time taken between receipt in the laboratory and processing is largely irrelevant and meaningless as it doesn't take into account when the sample was taken/transport times. A transport time of two to four hours plus up to another two hours to process a sample is not going to yield accurate results. Increasing the time for samples to be processed in the laboratory from two to four hours is an astounding change given the evidence for decline in WBC with time (you already quote evidence for this in the old document). If you want to promote quality/accuracy, then I can't see that you have any choice other than to recommend that samples are processed within one hour of the sample being taken and certainly within two hours as recommended by eg the EFNS task force. I don't have access to CLSI documents, but I believe they also recommend this.			
Evidence			
Your own evidence stated in the original document.-Guidelines on routine CSF analysis. Report from an ENFS task force. Europ. J. Neurol. 2006; 13:913-22.-Effect of delay in analysis of CSF parameters. Arch Dis Child Feta Neonatal Ed 2010;95:25-29			
Recommended Action	**ACCEPT** Document has been amended and brought in to line with B 37.		

Comment Number	3		
Date Received	10/12/2012	**Lab Name**	Virus Lab, Aberdeen Royal Infirmary
Section	Introduction, top of page 8		
Comment			
I am pleased you have put aseptic in brackets of inverted commas, other than the 1st word on this page. Suggest delete aseptic here.			
Recommended Action	**NONE** This change has already been made.		

Comment Number	4		
Date Received	07/12/2012	**Lab Name**	Sunderland Royal Hospital
Section			
Comment			
It is about the reporting of the WBC count in CSF, CAPD, peritoneal fluid and other fluids. For blood specimens the WBC is always reported as x 10^9/L as by standard practice and in line with the Pathology Harmony recommendations. see http://www.pathologyharmony.co.uk/ But how do you report the WBC count for other body fluids such as CSF or peritoneal fluid? Currently the National Microbiology SOPs for CAPD fluids (UK Standards for Microbiology Investigations B 25) as well as the national SOP for CSF investigations (UK Standards for Microbiology Investigations B 27) as well as the national SOP for sterile fluids (UK Standards for Microbiology Investigations B 26) all relate to WBC counts expressed as x 10^6/L. Thus a CSF is abnormal if the WBC is > 5 x 10^6/L or > 0.005 x 10^9/L (same result in different units) but we (humans) tend to handle and understand better integer numbers rather than decimal numbers, thus reporting as x 10^6/L seems preferable to me. Our Microbiology lab uses x 10^6/L in these reports but we are merging with another lab using x 10^9/L: they want us to change! Can I assume that a lot of thinking as gone into the UK Standards B 25, B 26 and B 27 and thus there is no plan to harmonise WBC reports from body fluids to a different unit?			
Recommended Action	**NONE** There are no plans to change the UK SMIs at this time.		

Comment Number	5		
Date Received	06/12/2012	**Lab Name**	Clyde Microbiology Laboratory
Section	Microscopy 2.4.1 b		
Comment			
a. Use of white cell diluting fluid is used where there are a reasonable red blood cell count, not only when heavily bloodstained. Unless there is a thought that this also destroys the white cells. b. Also 2 hours can be an impossible time-frame to receive CSFs for microscopy in this era of merged labs.			
Evidence			
Samples to travel 25 miles by porter, taxi and porter to lab - may well be >2 hours. Transport even within sites can be a challenge and some samples can be deemed none emergency by clinician.			

Recommended Action	a. **ACCEPT** The word heavily will be removed from the document. b. **NONE** These are high priority samples.

Comment Number	6		
Date Received	05/12/2012	**Lab Name**	Belfast HSCT
Section	General		
Comment			
I think the SMI can be less congested and simpler by making a separate SMI for the investigation of mycobacteria plus or minus other rare pathogens.			
Recommended Action	**NONE** These organisms are mentioned only for background and are covered in more depth in other documents.		

Comment Number	7		
Date Received	04/12/2012	**Lab Name**	EUROIMMUN UK Ltd.
Section	General		
Comment			
The documents contain no reference to antibody diagnostics of CSF/serum pairs in accordance with the recommendations of Reiber, which provide decisive guideleines on the diagnosis of inflammatory process in the CNS. The significance of CSF diagnostics has been described in various publications of Prof. Reiber.			
Evidence			
For example: Reiber H (1994) Flow rate of cerebrospinal fluid (CSF) a concept common to normal blood-CSF barrier function and dysfunction in neurological diseases. J Neurol Sci 122:189 203 Reiber H (1995a) External quality assessment in clinical neurochemistry: Survey of analysis for cerebrospinal fluid (CSF) proteins based on CSF/serum quotients. Clin Chem 41:256 263 Reiber H, Lange P (1991) Quantification of virus-specific antibodies in cerebrospinal fluid and serum: Sensitive and specific detection of antibody synthesis in brain. Clin Chem 37:1153 1160 Reiber H, Peter JB (2001) Cerebrospinal fluid analysis disease-related data patterns and evaluation programs. J Neurol Sci 184:101			
Recommended Action	**PARTIAL ACCEPT** The scope of this document will be amended to make it clear that viruses and immunological conditions are not covered.		

Comment Number	8		
Date Received	30/11/2012	**Lab Name**	Great Ormond Street Hospital
Section	2.5.2 2.5.3		
Comment			
Broth culture should be used for neurosurgical infections, especially when antimicrobials have already been started and to investigate late shunt related infection. The use of a 7 day FAA plate may be insufficient to detect the slow growing propionibacteria in shunt related infections; minimum 14 day incubation should be advised, but ideally there should be a prolonged anaerobic enrichment broth and then subculture.			
Evidence			
HPA Standard protocol recommends- a primary anaerobic plate for 7 - 14 days (and then says read at 40hr and 5 days, so that is a bit confusing). - but no broth but I am not convinced by that advice. I quite see the rational for no broth in non-neurosurgical infection (Shah SS PIDJ 2012 - Cerebrospinal fluid enrichment broth cultures rarely contribute to the diagnosis of bacterial meningitis). However the two references quoted both actually recommend broths in shunt CSFs (Meredith F 1997, and Dunbar SA 1998)- Meredith FT J Clin Micro 1997. Clinical Utility of Broth Cultures of Cerebrospinal Fluid from Patients at Risk of Shunt Infection Concluded: 'suspected CSF shunt infection may be one of the few remaining clinical scenarios in which the use of a broth medium for culture may be helpful to the clinician. Consequently, we recommend the continued use of broth medium for the culture of CSF from patients with CSF shunts to exclude the possibility of an infection caused by Propionibacterium sp.'- Dunbar SA 1998 J Clin Micro 1998 Microscopic Examination and Broth Cultures of Cerebrospinal Fluid in Diagnosis of Meningitis Concludes that: CSF specimens should be cultured in broth in special cases only, such as patients with CNS shunts....The more recent published literature on culture of Propionibacterium from shunts (or prosthetic joints) supports prolonged anaerobic culture eg Kai Arnell et al Journal of Neurosurgery: Pediatrics May 2008 / Vol. 1 / No. 5 / Pages 366-372 Cerebrospinal fluid shunt infections in children over a 13-year period: anaerobic cultures and comparison of clinical signs of infection with Propionibacterium acnes and with other bacteria. Which says: The addition of cultures for anaerobic bacteria and prolonged observation time of the cultures led to an increase in the diagnostic yield by more than one third. Infection with P. acnes resulted in a mild clinical picture that may easily be overlooked if adequate anaerobic cultures are not obtained.Likewise culture from PJI suggest the same eg Butler-Wu J Clin Micro 2011 Optimization of Periprosthetic Culture for Diagnosis of Propionibacterium acnes Prosthetic Joint Infection In our own experience, we introduced the Robertson's Cooked Meat extended incubation (5-7 days) and anaerobic subculture (further 7 days) a few years ago and find Propionibacterium in the broth that was not on the 5 day anaerobic plate and we feel are clinically significant. (The majority of broths are no growth, even setting up on the open bench).This may partially explain the higher % yield of GPRs between a historical (non-impregnated shunts) 1993 - 2003 cohort compared to a recent Bactiseal (clindamycin and rifampicin impregnated) cohort, and lower yield of 'no growths' treated as infection; possibly the antimicrobial impregnated shunt has 'uncovered' a cohort of late GPR infection by reducing the CoNSs. Organisms isolated from shunt CSF infections at GOSH 1993-2003-1592 shunts (non bactiseal)- 8.4% infection rate; 133 infections, only 2 propionibacteria but 7 no growth (treated as			

infection) 499 recent bacteseal shunts with extended anaerobic culture- 5% infection; 25; 4 propionibacteria/other GPR and 1 no growth. Hence, I would suggest extended anaerobic culture as standard minimum 14 days ideally with a broth enrichment first. Additionally, if not prepared to use a broth in all, I would recommend essential if antimicrobials have already been started (no evidence started, but good common sense and clinical experience!)	
Recommended Action	**ACCEPT** With a caveat that they are only useful in certain circumstances.

Consultation: 18/03/2013 – 07/06/2013

Version of document consulted on: B 27df+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	18/03/2013	**Lab Name**	Microbiology, Pilgrim Hospital, Boston, Lincs
Section			
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?			
Yes. This is a reasonable time scale to allow for growth of slow-growers and is in line with yeast/fungal culture incubation times set out in other UK SMI's.			
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?			
Yes. However, in Section 3.1.1 the time quoted for microscopy reporting is 2 hours. This is too long. I would take a serious look at a laboratory organisational and prioritisation structure that allowed two hours for such an important result to be produced. A 1 hour microscopy reporting time would seem much more reasonable and should be easily achievable by a well organised laboratory with competent staff.			
Do you have any views on the use of broth cultures for diagnosing shunt infections?			
No.			
Recommended Action	N/A		

Comment Number	2		
Date Received	05/04/2013	**Lab Name**	Microbiology Department, Freeman Hospital, Newcastle Hosp Trust

Section	2.7 and 2.8
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?	
Our laboratory protocol is 10 day incubation for extended anaerobic culture and 21 days for extended fungal investigations.	
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?	
Yes.	
Do you have any views on the use of broth cultures for diagnosing shunt infections?	
Not routinely performed; may be performed as the request of microbiologist.	
Comment	
a. 2.7 - Please consider inclusion of referral to EUCAST guidance as well as BSAC b. 2.8 - Web link to the reference lab user manual and request forms is a very useful inclusion	
Recommended Action	a. **ACCEPT** This is being done as part of the transfer to the PHE template. b. **NONE**

Comment Number	3		
Date Received	24/04/2013	**Lab Name**	Nottingham
Section	2.5.3		
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?			
No What is the evidence for 14 days, aware of extended incubation until 10 days for more fastidious and Proprionobacteria. Beyond that will the plates still be moist enough for organisms to grow?			
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?			
Yes.			
Do you have any views on the use of broth cultures for diagnosing shunt infections?			
Aware may increase yield, but difficult to interpret and distinguish from contamination during sampling and processing if broth only positive.			
Comment			
Table 2.5.3 For the neurosurgical samples it's unclear of what point extended plates are read and results reported. As FAA plate incubated for 14 days but states read >40hr and 5 days			

Recommended Action	ACCEPT UK SMI amended to make clearer and amended to ten days.

Comment Number	4		
Date Received	03/06/2013	Lab Name	Western Sussex Hospitals Microbiology Laboratory
Section			
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?			
Yes.			
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?			
Yes.			
Do you have any views on the use of broth cultures for diagnosing shunt infections?			
Should be used - original paper specified whilst not useful in general they were for abscesses and shunt infections. J Clin Microbiol 1997;35:3109-11. Consequently, we recommend the continued use of broth medium for the culture of CSF from patients with CSF shunts to exclude the possibility of an infection caused by Propionibacterium sp.			
Comment			
I would have liked to see a worked example of calculating an uncertainty of Measurement for the cell count.			
Recommended Action	**NONE** This comment will be considered as part of the review of the Quality SMI documents in 2015.		

Comment Number	5		
Date Received	06/06/2013	Lab Name	Kingston Hospital
Section			
Comment			
a. The CSF WCC reference ranges for neonates and age 1- 4 appear higher than most paediatric reference ranges. They are also contradictory to the figures proposed in the NICE 2010 Meningitis Guidance for Children. May I know what is the rationale behind this? b. Also there is currently no CSF reference range for age between > 7 days to 12 months old. May I know why?			

Recommended Action	a. **ACCEPT** This section has been made clearer b. **NONE** A caveat has been added to say that the table is just for guidelines

COMMENTS RECEIVED OUTSIDE OF CONSULTATIONS

Comment Number	1		
Date Received	11/09/2012	**Lab Name**	MSTAG
Section	a. General Comment b. Normal CSF values table c. 2.7 d. 2.5.3 e. 2.4.1 f. 2.1 g. 1.4 h. Abnormalities assoc. with bacterial meningitis.		
Comment			
a. Would like to see more detail about TB meningitis. b. Clarify neonate age range, specify type of luecocoytes. c. BSAC guidelines given, but many labs are now using other guidelines. d. First part of table clinical details etc. instead of giving list of clinical details how about stating ALL CSFs in this section get a minimum of Choc and BA Immunocompromised patients up to 8 weeks for SAB plate disputed, suggest add comment incubate up to 14 days if indicated FAA/NEO incubation time given as 7-14 d, but next column-cultures read gives 40hr and 5d. Suggest 5 days. e. Section 2-has a chunk of text been removed? It doesn't make sense. Gram stain: suggest simplify this section-instead of listing exclusions, how about something along the lines of: 'Perform Gram stain on neonates, Immunocompromised and raised cell counts'.The word sterile is used in this section regarding centrifuging in a sterile capped conical container. Clotted specimens-describes how to make a smear for Gram stain but in previous section states do not perform Gram. f. Paragraph 5-either provide more detail for TSE agents (lots of……..dots used) or provide a reference. Subsequent paragraphs regarding processing under a hood-is this the recommendation for ALL CSF samples? g. 10ml for Mycobacteria! This is an extremely large volume for CSF, the MRU recommend a minimum of 0.5ml. h. Paragraph 3 WBC: RBC ratio-would be clearer if this was added to the above table and age ranges, given ie the gap between newborn and adult is a large			

range.	
Recommended Action	a. **NONE** This is covered in more detail in B 40. b. **PARTIAL ACCEPT** Document updated. c. **NONE** The group has agreed to continue to recommend BSAC until such a time as they become EUCAST. d. **NONE** All UK SMIs start with clinical details and most users find this the most useful presentation. e. **NONE** This change has already been made. f. **NONE** This change has already been made. g. **ACCEPT** The document has been updated. h. **NONE** Information in table is standard for this area.

Comment Number	2		
Date Received	03/01/2013	**Lab Name**	Southampton General Hospital
Section	SOP numbers B 22 and B27 both concerning CSF and CSF shunts		
Comment			
I have just had one of our consultants ask why we weren't following these SOPs. We have found conflicting and confusing information. For the investigation of CSF culture the incubation times states 7 to 14 days but only states to read at >40hrs and at 5 days with no mention of 14 day reading anywhere. Please could this be clarified for us. We have only followed the reading and reporting at 5 days but will change as soon as this is understood.			
Recommended Action	**ACCEPT** This section of the document has been amended to make it clearer.		

Comment Number	3		
Date Received	02/01/2013	**Lab Name**	University Hospitals of Leicester NHS Trust
Section	Whole document		
Comment			
a. Do you know if the advice re neurosurgical type CSFs and anaerobic incubation and when you read the plates, and use of broth enrichment is going to change? b. Currently it states incubation for 7-14 days but only read at 40hr and 5 days, which is a bit confusing! c. It also doesn’t mention enrichment broths, is this likely to change for this type of CSF?			
Recommended Action	a. **ACCEPT** When plates are read has been made clearer. The advice on broths remains the same. b. **ACCEPT** When plates are read has been made clearer. c. **PARTIAL ACCEPT** Broths are mentioned in the document as useful in certain circumstances, this will be strengthened.		

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 1			
Date Received	29/05/2013	**Lab Name**	Golden Jubilee National Hospital

UK Standards for Microbiology Investigations

Investigation of Genital Tract and Associated Specimens

NICE accredited
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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 28 | Issue no: 4.6 | Issue date: 26.04.17 | Page: 1 of 40

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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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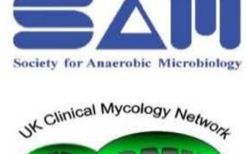

Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 7

SCOPE OF DOCUMENT 9

SCOPE 9

INTRODUCTION 9

TECHNICAL INFORMATION/LIMITATIONS 20

1 SAFETY CONSIDERATIONS 22

2 SPECIMEN COLLECTION 22

3 SPECIMEN TRANSPORT AND STORAGE 24

4 SPECIMEN PROCESSING/PROCEDURE 24

5 REPORTING PROCEDURE 32

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 34

REFERENCES 35

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	10/26.04.17
Issue no. discarded.	4.5
Insert Issue no.	4.6
Section(s) involved	**Amendment**
Page 32.	Change to the scoring for *Mobiluncus* morphotypes to match ref. 72 (>30/hpf =**2** not 4; 5-30/hpf =**2** not 3; 2-4/hpf =**1** not 2).
Page 33.	Change to the interpretation of the scoring to match ref. 72 (Abnormal: indicative of BV when Total score ≥**7** not 6).

Amendment No/Date.	9/08.12.14
Issue no. discarded.	4.4
Insert Issue no.	4.5
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Reporting Procedure.	Due to a transcription error during transfer to the PHE template, some text regarding Nugent's criteria score was removed. This information has been re-instated in the document.

Amendment No/Date.	8/24.04.14
Issue no. discarded.	4.3
Insert Issue no.	4.4
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template

	to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	7/18.12.12
Issue no. discarded.	4.2
Insert Issue no.	4.3
Section(s) involved	**Amendment**
Whole document.	Minor formatting amendments.
2.5.3.	Section number amended in table.

Amendment No/Date.	6/11.07.12
Issue no. discarded.	4.1
Insert Issue no.	4.2
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC1,2. Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.

References.	Some references updated.

Amendment No/Date.	5/03.05.05
Issue no. discarded.	4
Insert Issue no.	4.1
Section(s) involved	**Amendment**
Front page.	Redesigned.
Status of document.	Reworded.
Amendment page.	Redesigned.

Amendment No/Date.	4/15.12.03
Issue no. discarded.	3.1
Insert Issue no.	4
Section(s) involved	**Amendment**
Whole document.	Text revision.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

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laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2017). Investigation of Genital Tract and Associated Specimens. UK Standards for Microbiology Investigations. B 28 Issue 4.6. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of Document

Type of Specimen

High vaginal swab (HVS), vaginal discharge, vulval swab, labial swab, cervical swab, endocervical swab, penile swab, urethral swab, genital ulcer swab, semen, screening swabs for *N. gonorrhoeae,* aspirates from bartholin's gland, fallopian tube, tubo-ovarian abscess, pouch of Douglas fluid, intra-uterine contraceptive device (IUCD), products of conception

Scope

This SMI describes the examination of genital specimens for the presence of Neisseria gonorrhoeae, yeasts, -haemolytic streptococci and other specific target organisms (including Trichomonas vaginalis), and the microscopic diagnosis of bacterial vaginosis (BV).

This SMI should be used in conjunction with other SMIs.

Introduction

Appropriate specimens are often difficult to obtain, particularly from women, and incorrect or sub-optimal specimens are often received. It is important to avoid contamination with faecal flora during collection of specimens.

This SMI is laid out under the following headings:

Sexually transmitted infections (STIs).

Vaginal infections other than STIs.

Other infections of the female genital tract.

Infections (other than STIs) of the male genital tract.

Sexually Transmissible Infections

A range of sexually transmissible organisms cause infections responsible for a large number of clinical syndromes. When a specific STI is diagnosed, it is recommended to screen for other infections. Screening has a role in helping to control gonorrhoea, syphilis, chlamydial infection, and human immunodeficiency virus (HIV) infection.

Gonorrhoea[1,2]

N. gonorrhoeae causes a wide spectrum of clinical syndromes in men, women and neonates infants.

Local gonococcal infections in men

Local gonococcal infections in men most commonly present as symptomatic urethritis with a purulent urethral discharge and dysuria. The most common complication of this is acute epididymitis and, in rare cases, gonococcal urethritis can be complicated by gonococcal cellulitis, penile lymphangitis, or periurethral abscess.

Proper specimen collection is important to ensure optimal yield. The best specimen is expressed urethral exudate. In asymptomatic men a urethral swab is taken.

Local gonococcal infections in women

Local gonococcal infections in women primarily affect the cervix. Gonococcal cervicitis is often asymptomatic, but it can cause an increased vaginal discharge, genital itching or dysuria. The urethra is frequently involved in women who have had a hysterectomy. The most important complication of gonococcal infection in women is pelvic inflammatory disease (PID), which may lead to infertility. Endocervical or urethral swabs are the preferred specimens.

Anorectal gonorrhoea

Anorectal gonorrhoea may be an asymptomatic complication in women with cervical gonorrhoea. Women and homosexual men who participate in receptive anal intercourse with infected partners are at risk of developing anorectal gonorrhoea. Most men with anorectal gonorrhoea are asymptomatic though some develop symptomatic proctitis. Homosexually active men with symptomatic proctitis can be infected with a variety of other pathogens. Rectal specimens may be taken to seek *N. gonorrhoeae, Chlamydia* trachomatis and viruses[3].

Asymptomatic mucosal infection

Asymptomatic mucosal infection can occur at any mucosal site such as the urethra, cervix, rectum and pharynx. Such infections are detected by screening patients presenting with appropriate history, symptoms and signs suggesting exposure. Throat swabs are taken for screening for *N. gonorrhoeae* if there is a history of orogenital contact.

Disseminated gonococcal infection (DGI)

Disseminated gonococcal infection (DGI) is a rare complication of gonorrhoea. This presents with one or more of the following[4]:

- arthralgia (joint pain)
- asymmetric polyarthritis
- rash (often pustular)
- myalgia (muscle pain)
- septic arthritis
- tenosynovitis (inflammation of a tendon sheath)

In most cases of DGI, mucosal infection is present but can be asymptomatic. All potential mucosal sites should be screened for *N. gonorrhoeae*. Blood cultures and examination of fluids such as joint fluids may be useful in diagnosis. Dermatitis as a result of DGI is characterised by a small number of skin lesions that are located mainly on the extremities. DGI may be complicated when seeding of the heart valves or meninges results in gonococcal endocarditis or meningitis. The most common manifestation of DGI is the arthritis-dermatitis syndrome[5]. DGI resulting in meningitis is very rare[6].

Sexual transmission of *Neisseria meningitidis* may also cause lower genital infections in both men and women, but asymptomatic colonisation usually results.

Media for the isolation of *N. gonorrhoeae* may become overgrown with yeasts. In addition, it has been demonstrated that *C. albicans* may produce a soluble factor which may inhibit the growth of *N. gonorrhoeae*[7]. Therefore, this SMI recommends the use of selective media containing antifungal agents.

Trichomoniasis[8]

Trichomoniasis is caused by the flagellate protozoan, *T. vaginalis*; it is almost always acquired through sexual contact. Presenting symptoms include an increased vaginal discharge, pruritus and dysuria. An erythematous, friable cervix with punctate areas of exudate (strawberry cervix) is pathognomonic of *T. vaginalis*. Long-term carriage may occur, with symptoms not appearing for years after the initial sexual contact. *T. vaginalis* infection in pregnancy has been associated with low birth weight and preterm delivery[9]. The prevalence of *T. vaginalis* remains at a constant low level in cases seen in GUM clinics[10].

Various techniques including culture followed by microscopy, direct microscopy and immunodiagnostic methods for the detection of *T. vaginalis* have been compared[11-15]. Microscopy has been the most practicable means of diagnosis for routine screening[16,17]. Microscopy of a wet preparation is highly specific and easily performed, but it fails to detect 30-50% of *T. vaginalis* infections (even when undertaken close to the patient) compared to culture which is regarded as the 'gold standard'[12,13]. Detection using films stained with acridine orange has been found to be only slightly more sensitive than unstained wet preparations[14].

Conventional culture methods are slow and labour-intensive, but a method utilising microtitre trays read with an inverted microscope has been described which is cost-effective, without any loss in sensitivity[13].

This SMI recommends selective culture from patients with clinically suspected *T. vaginalis* infection, with other diagnosed or suspected STI, in pregnancy, when requested and in other groups according to local protocols. Local protocols may vary depending on local prevalence.

Genital ulcers

Genital ulcers are most commonly caused by[18]:

- Herpes simplex virus (HSV)
- *C. trachomatis* (lymphogranuloma venereum)
- *Treponema pallidum* (syphilis)
- *Calymmobacterium granulomatis* (granuloma inguinale)
- *Haemophilus ducreyi* (chancroid)

Investigations for HSV, *T. pallidum* and *C. trachomatis* are not covered by this SMI.

Staphylococcus aureus and Lancefield group A streptococci may also cause tender pustules resembling ulcers on genitalia, as well as inguinal lymphadenopathy and soreness[18].

Chancroid

Chancroid is an important cause of genital ulceration in the tropics, and its incidence increased dramatically in North America during the late 1980s. It is caused by *H. ducreyi* which enters via a break in the epithelium[19]. Chancroid ulcers are vascular, painful and the granulomatous base bleeds easily.

Lesions occur on and around the genitalia[18,20]. As well as genital ulcers, painful inguinal lymphadenopathy (buboes) can develop in about 50% of cases[19].

Asymptomatic carriage appears to be rare. Infection rarely presents as urethritis alone without any genital ulcers[21].

The incidence of chancroid is reportedly increasing in many areas, although diagnosis is often made on clinical grounds alone and may thus be inaccurate[19]. Chancroid, in common with other sexually transmitted diseases, is thought to be an important co-factor in the transmission of HIV in the tropics[22].

Examination of Gram stained material from genital ulcers has poor sensitivity and specificity[19,20]. Results from immunofluorescence and molecular techniques are encouraging but need further evaluation[23].

Isolation of *H. ducreyi* is comparatively difficult and requires selective agar media, although isolation rates of up to 80% have been reported[19,22-24].

C. granulomatis[25]

C. granulomatis infection is a rare condition found only in certain parts of the tropics. This organism has rarely been grown *in vitro* and culture is not routinely practicable. It is demonstrated by performing Giemsa or Wright stains on scrapings from the edge of the ulcer. It is an encapsulated Gram negative bacterium. The organisms or "Donovan bodies" appear as a cluster of blue or black bodies with a "safety pin" morphology found within PMNs. The primary lesion begins as an indurated nodule that erodes to form a granulomatous, heaped ulcer. Lesions occur on the folds of the scrotum, thighs, labia and vagina.

Genital warts

Genital warts is a venereal infection caused by human papillomavirus (HPV)[26]. Sub-clinical carriage of HPV is common and greatly exceeds the prevalence of visible warts[27]. They may occur as flat warts, which may progress to carcinoma *in situ*, or may occur as a papillary projection above the skin with a rich capillary bed. In women, warts are located most frequently in the posterior introitus and labia and less commonly in the perianal area. In uncircumcised men the prepuce is the most common site of infection[27].

Children are also at risk of acquiring sexually transmissible infections[28-34]. Although the presence of a sexually transmitted organism beyond the neonatal period is highly suggestive of sexual abuse, and this possibility should always be investigated, exceptions do exist. Rectal or genital infection with *C. trachomatis* among young children may be the result of prenatally acquired infection and may persist for as long as three years. Similarly, anogenital warts may be present in pre-pubertal children as a consequence of prenatal transmission, or of autoinoculation from common hand warts. Bacterial vaginosis has been identified among both abused and non-abused children.

Specimens for forensic or medico-legal investigations are outside the remit of this SMI, and should be processed according to local protocols. It is advisable to use a ‘chain of evidence’ procedure when processing specimens from possible cases of sexual assault or abuse. In such cases, appropriate specimens may be taken for investigation for *C. trachomatis, N. gonorrhoeae*, and the presence of *T. vaginalis* and clue cells[35].

Vaginal Infections (other than STIs)

Normal vaginal flora

Normal vaginal flora consists of a wide range of organisms including *Lactobacillus*

species, streptococci, enterococci and coagulase negative staphylococci[36-39]. Anaerobes, such as *Bacteroides* species and anaerobic cocci, *Gardnerella vaginalis*, yeasts, coliforms, *Ureaplasma urealyticum* and *Mycoplasma* species may also be present as part of the normal flora, but they have also been incriminated in vaginal infections.

Vaginal candidosis

Vaginal candidosis occurs when alterations in the vaginal environment allow yeasts (which are often present as commensal organisms in the vagina), to proliferate. Increased levels of oestrogens promote their growth. Yeast overgrowth is often seen in the following conditions:

- after antimicrobial therapy
- diabetes mellitus
- immunosuppression
- obesity
- pregnancy
- use of oral contraceptives

Although *Candida albicans* is isolated in 80-90% of cases of vaginal candidosis, other yeasts account for 10-15% of cases and include[40]:

- *C. krusei*
- *C. kefyr*
- *C. tropicalis*
- *C. glabrata*

Cases commonly present with pruritus, dysuria and a whitish discharge, although sometimes there is just mucosal erythema and soreness. Infections with species other than albicans may result in treatment failure and subsequent persistent infections.

This SMI recommends routine culture of all vaginal, endocervical and urethral swabs for yeasts.

Vaginitis[41]

Vaginitis can be caused by *Candida* species and *T. vaginalis*. In children, infections caused by β-haemolytic streptococci and *S. aureus* are common[42]. Lancefield group A streptococci also cause vaginitis and purulent vaginal discharge in adults[43].

Atrophic vaginitis is a rare condition usually associated with the elderly[44]. The majority of women with mild to moderate atrophy are asymptomatic. Reduced endogenous oestrogen causes the epithelium to thin, contributing to a reduction in lactic acid production and an increase in vaginal pH. This change causes overgrowth with mixed flora and the disappearance of lactobacilli. The vaginal discharge contains polymorphonuclear leucocytes and small round basal epithelial cells.

Vulvovaginitis

Vulvovaginitis is mainly seen in pre-pubertal females, but may affect women of any age. It may be associated with poor hygiene, skin irritation due to soaps, or with

streptococcal throat carriage. Symptoms include irritation, soreness and discharge. Causative organisms include[18,45-47]:

- Lancefield group A streptococcus
- *Staphylococcus aureus*
- *C. albicans*
- *Haemophilus influenzae*
- *N. gonorrhoeae*

Other unusual organisms may cause vulvovaginitis, including *Salmonella* and *Shigella* species[48]. Threadworm infestation may predispose to vulvovaginitis (see B 31 – Investigation of Specimens other than Blood for Parasites).

Bacterial vaginosis (BV)

Bacterial vaginosis (BV) is characterised by an increase in anaerobes and a decrease in *Lactobacillus* species[49,50].

BV has been regarded in the past as a harmless abnormality. However, it is now considered to be associated with a variety of genital tract infections and complications including[49,51]:

- amnionitis
- postpartum endometritis and fever
- preterm labour and low birth weight
- premature rupture of membranes (PROM)
- post vaginal hysterectomy sepsis
- pelvic inflammatory disease (PID)
- urinary tract infections
- BV may be diagnosed clinically if three of the following four criteria are fulfilled[52]:
    - grey-white, thin homogenous discharge
    - vaginal secretions pH > 4.5
    - positive amine odour test (release of fishy amine odour when vaginal secretion is mixed with 5-10% potassium hydroxide)
    - presence of clue cells on microscopic examination

A normal vaginal flora is associated with the presence of *Lactobacillus* species alone, or in the presence of small numbers of *G. vaginalis* morphotypes. The shift in vaginal flora associated with BV is characterised by a decrease in numbers of lactobacilli which are replaced by a mixed flora of aerobic, anaerobic and microaerophilic species[53]. A diverse group of organisms is involved, many of which are difficult to grow. Organisms associated with BV include[49-51,53]:

- *Prevotella* species
- *G. vaginalis*
- *Mobiluncus* species

- *Peptostreptococcus* species
- *Mycoplasma hominis*

Although *G. vaginalis* is encountered consistently and in large numbers in women with BV, the organism can also be isolated from as many as 60% of asymptomatic women. Direct examination of vaginal secretions is more relevant for the diagnosis of BV than is the isolation of *G. vaginalis* from these specimens[54].

Examination of Gram stained films is reported to be useful in the diagnosis of BV and standard criteria for morphotypes have been described[50,54,55]. In typical smears from patients with BV, clue cells are accompanied by a mixed flora consisting of very large numbers of small Gram negative rods (predominantly *Prevotella* species) and Gram variable rods and coccobacilli (predominantly *G. vaginalis*) in the absence of larger Gram positive rods (*Lactobacillus* species). Curved Gram variable rods (*Mobiluncus* species) may also be present.

Clue cells are epithelial cells to which Gram variable rods are attached in large numbers, obscuring the cell border. They are reported as being highly specific (almost 100%), but not as sensitive as using other aspects of a Gram stain to detect BV[56]. Using Amsel's original criteria, clue cells are only significant if two of the other three criteria (grey-white discharge, pH >4.5 and a positive amine test) are fulfilled.

Gram staining (using the criteria of Nugent or Hay) of vaginal smears is the most sensitive method for the laboratory diagnosis of BV as it detects both clue cells and the disturbance in bacterial morphotypes associated with BV[50,52,54,55]. It is not necessary to see clue cells to make a diagnosis of BV. One of the key features is the absence of typical lactobacilli and their replacement with Gram variable or Gram negative rods[39]. However, one study found that acridine orange or wet preparations are more sensitive methods for detecting clue cells than the Gram stain[57]. Detection of clue cells alone, whilst highly specific for BV, is not as sensitive as detection of different morphotypes by the Gram stain[56].

This SMI recommends the examination of all vaginal swabs from women of child bearing age for the presence of BV by Gram film.

Toxic shock syndrome (TSS)

Toxic shock syndrome (TSS) is an acute multi-system illness characterised by fever, hypotension, erythematous rash, diarrhoea and desquamation of the skin upon recovery[58]. TSS is caused by a toxin produced by *S. aureus*. Isolation of a toxin-producing *S. aureus* from a mucous membrane is strong support for a positive diagnosis. There is a TSS-like illness caused by Lancefield group A streptococci.

TSS can be associated with:

- tampon use
- childbirth or other surgical wound infection
- contraceptive devices
- cervico-vaginal colonisation with *S. aureus*

Lancefield group B streptococcus

Lancefield group B streptococcus normally colonises the vagina in many women. In pregnancy this organism can infect the amniotic fluid (see B 26- Investigation of Fluids

from Normally Sterile Sites) which can lead to neonatal sepsis, pneumonia and meningitis. According to local protocol, patients judged at high risk for the development of group B streptococcal infection may be screened for carriage. Optimum yield will be achieved by selective/enrichment procedures applied to swabs obtained from the vagina and the anorectum[59-61].

Conditions considered to confer a high risk of infection include:

- fever in labour
- premature labour
- premature rupture of membranes (PROM)
- previously infected baby

Listeria monocytogenes

Listeria monocytogenes may cause serious infection in pregnant women, neonatal infants and patients who are immunocompromised[62,63]. In pregnant women septicaemia caused by *L. monocytogenes* presents as an acute febrile illness that may affect the fetus[62]. This may lead to systemic infection (granulomatosis infantisepticum), stillbirth and neonatal meningitis. Products of conception, placenta and neonatal screening swabs should be examined for this organism. Routine culture of vaginal swabs for *L. monocytogenes* is not usually performed although it may be useful in suspected cases[64]. Blood cultures are indicated. Serological investigations have no place in the diagnosis of listeriosis[63].

Septic abortion

Septic abortion may result in serious maternal morbidity and may be fatal[62]. Uterine perforation, presence of necrotic debris, and retained placental products can lead to infection. Most infections are polymicrobial and involve anaerobes.

Clostridial sepsis complicating abortion is potentially lethal. *Clostridium* species are part of the normal vaginal flora in some women.

Other Infections of the Female Genital Tract

Bartholinitis[62]

Bartholinitis is inflammation of the Bartholin glands, the small mucus-producing glands on each side of the vaginal orifice of adult women. Two stages of infection occur. The first stage is acute infection of the duct and lining of the gland. The second stage is abscess formation in which the gland is obstructed.

Causative organisms of Bartholin's gland infections include[62,65]:

- *anaerobes*
- *N. gonorrhoeae*
- *streptococci*
- *Enterobacteriaceae*
- *C. trachomatis*
- *H. influenzae*
- *S. aureus*

- *other Neisseria* species
- *M. hominis*

Mucopurulent cervicitis

Mucopurulent cervicitis is inflammation of cervical columnar epithelium. Causative organisms include[41]:

- *C. trachomatis*
- HSV
- *N. gonorrhoeae*

Other organisms such as *U. urealyticum*, *M. hominis* and those linked with BV have not been consistently associated with mucopurulent cervicitis, suggesting only a weak association or their dependence on the presence of other organisms[41].

Cervicitis is important as it provides a source of pathogenic organisms which may infect the endometrium and endosalpinx. Ascent during pregnancy can cause chorioamnionitis, premature rupture of membranes, puerperal and neonatal infections.

Gram stained smears are used to evaluate the presence of polymorphonuclear leucocytes[66]. Mucopurulent cervicitis is characterised by the presence of an endocervical exudate containing PMNs. A visible yellow discharge is produced.

Endometritis

Endometritis is inflammation of the endometrium, the inner lining of the uterus. Organisms that may cause this infection include:

- *C. trachomatis*
- *N. gonorrhoeae*
- *Mycobacterium tuberculosis*
- HSV

Postpartum endometritis

Postpartum endometritis - most infections are caused by vulvovaginal flora that ascends into the uterus. Infections are often polymicrobial and caused by[67]:

- β-haemolytic streptococci
- *S. aureus*
- enterococci
- anaerobes
- *C. trachomatis*
- Enterobacteriaceae
- *G. vaginalis*
- *M. hominis*

Risk factors include:

- amniotic fluid infection

- caesarean delivery
- invasive foetal monitoring
- prolonged rupture of membranes
- vaginal examinations

Appropriate specimens include a swab of the lower uterine segment or the cervix.

Salpingitis

Salpingitis is inflammation of the uterine (fallopian) tube. Infection is sometimes polymicrobial involving[68]:

- *C. trachomatis*
- *N. gonorrhoeae*
- mixed anaerobic, facultative anaerobic and aerobic bacteria
- *M. hominis*

Specimens from the fallopian tubes are superior to endocervical swabs. Endocervical swabs may be useful but require more careful interpretation. Acute salpingitis can result in sequelae such as chronic abdominal pain and an increased risk of ectopic pregnancy.

Pelvic inflammatory disease (PID)

Pelvic inflammatory disease (PID) is the term used to refer to endometritis, salpingitis, pelvic peritonitis or a combination of these. Symptoms include dyspareunia, intermenstrual bleeding and lower abdominal cramps.

Many women who develop PID suffer long term sequelae such as:

- chronic pelvic pain
- ectopic pregnancy
- Infertility
- pyosalpinx (collection of pus in a fallopian tube)
- tubo-ovarian abscess (TOA)

PID is often a polymicrobial illness[69,70]. Women with gonococcal PID may also be infected with *C. trachomatis.*

The preferred specimens for diagnosis of PID are aspirates collected from a fallopian tube or a TOA, or peritoneal fluid (processing peritoneal fluid is described in B 26 - Investigation of Fluids from Normally Sterile Sites). Swabs of pus or fluid are acceptable but where possible pus or fluid samples should be sent. These are processed in the same manner as pus/fluid.

Organisms that cause PID include:

- *C. trachomatis*
- *N. gonorrhoeae*
- anaerobes
- Lancefield group B streptococcus

- other streptococci
- *Escherichia coli*
- *G. vaginalis*
- *Actinomyces israelii*
- *M. hominis*
- *H. influenzae*

Intrauterine contraceptive devices (IUCDs)

Intrauterine contraceptive devices (IUCDs) - the presence of an IUCD may be associated with PID[71]. Infections may be polymicrobial with the isolation of both Gram positive and Gram negative aerobic and anaerobic organisms. *Actinomyces* species, particularly *A. israelii*, may be significant isolates. This SOP recommends that IUCDs are only cultured where there are clinical indications of PID or other inflammatory conditions.

Infections (other than STIs) of the Male Genital Tract

Prostatitis

Prostatitis is inflammation of the prostate. Acute or chronic infection may be caused by *Enterobacteriaceae, C. trachomatis, N. gonorrhoeae* and streptococci. *Cryptococcus neoformans* may be isolated from the prostate in patients who are HIV positive and this is an important site for persistence and a potential origin for relapse. Diagnosis is made by examining voided and midstream urine specimens as well as expressed prostatic secretions (see B 41 - Investigation of Urine).

Epididymitis

Epididymitis is inflammation of the epididymis. It may occur as a result of trauma or chemical irritation associated with urine reflux, or more usually as a complication of urethral or urinary infections. Diagnosis is usually made by examining urine or urethral swabs. Organisms that cause infection include[72]:

- *C. trachomatis*
- Enterobacteriaceae
- *N. gonorrhoeae*
- pseudomonads
- *M. tuberculosis*

Orchitis

Orchitis is inflammation of the testis. It is usually as a result of a blood-borne viral infection, the most common being mumps. Bacterial infection usually occurs as a result of contiguous spread. Diagnosis is made by examining urine. Causative organisms include[72]:

- Enterobacteriaceae
- pseudomonads
- staphylococci

- streptococci
- *M. tuberculosis*

Balanitis

Balanitis is inflammation of the glans penis[73].

Balanoposthitis

Balanoposthitis is inflammation of the prepuce and the glans penis.

Irritation due to smegma, urethral discharge or other agents can play a role in the aetiology of these two conditions.

Organisms that cause balanitis and balanoposthitis include[73-76]:

- yeasts
- HSV
- Lancefield group A streptococcus
- *S. aureus*
- Lancefield group B streptococcus.
- anaerobes

Candida

Candida species may be isolated in cases of penile thrush.

Urethritis in men is mainly caused by[72]:

- *N. gonorrhoeae*
- *C. trachomatis*
- *U. urealyticum*

Haemophilus species such as *H. influenzae* and *H. parainfluenzae* have been isolated from urethral discharge[77].

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[78,79]

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.”

1 Safety Considerations[78-94]

1.1 Specimen Collection, Transport and Storage[78-83]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[78-94]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[86].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

High vaginal swab (HVS), vaginal discharge, vulval swab, labial swab, cervical swab, endocervical swab, penile swab, urethral swab, genital ulcer swab, semen, screening swabs for *N. gonorrhoeae,* aspirates from Bartholin's gland, fallopian tube, tubo-ovarian abscess, pouch of Douglas fluid, intra-uterine contraceptive device (IUCD), products of conception

2.2 Optimal Time and Method of Collection[95]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[95].

Ideally, inoculation of specimens for *N. gonorrhoeae* is made directly to culture media at the bedside and incubated without delay. Transport time should be as short as possible.

For *H. ducreyi* direct inoculation of media ensures optimal recovery.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[96-100].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Genital tract swabs

Cervical and high vaginal swabs should be taken with the aid of a speculum. It is important to avoid vulval contamination of the swab. For *Trichomonas*, the posterior

fornix, including any obvious candidal plaques should be swabbed. If pelvic infection, including gonorrhoea, is suspected, the cervical os should be swabbed.

For the specific diagnosis of BV, it is recommended that an air-dried smear of vaginal discharge is sent in addition to the swab.

Separate samples should be collected into appropriate transport media for detection of viruses or *C. trachomatis.*

High vaginal swabs

After the introduction of the speculum, the swab should be rolled firmly over the surface of the vaginal vault. The swab should then be placed in Amies transport medium with charcoal[97].

Cervical swabs

After introduction of the speculum to the vagina, the swab should be rotated inside the endocervix. The swab should then be placed in Amies transport medium with charcoal[97].

Urethral swabs

Contamination with micro-organisms from the vulva or the foreskin should be avoided. Thin swabs are available for collection of specimens.

The patient should not have passed urine for at least one hour. For males, if a discharge is not apparent, attempts should be made to "milk" exudate from the penis. The swab is gently passed through the urethral meatus and rotated. Place the swab in Amies transport medium with charcoal[97].

Intrauterine contraceptive devices (IUCDs)

The entire device should be sent.

Rectal swabs

Rectal swabs are taken via a proctoscope.

Throat swabs

Throat swabs should be taken from the tonsillar area and/or posterior pharynx avoiding the tongue and uvula.

Fluids and pus

These are taken from the fallopian tubes, tubo-ovarian and Bartholin’s abscesses, etc… during surgery.

2.3 Adequate Quantity and Appropriate Number of Specimens[95]

Fluids and pus – preferably a minimum volume of 1mL.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage[78,79]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[95].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[95].

4 Specimen Processing/Procedure[78,79]

4.1 Test Selection

Investigation for *Chlamydia* and viruses may also be performed on genital tract specimens with the appropriate swabs and transport media for the organism under investigation.

Microscopy for BV

Either

A Gram stained film of the vaginal discharge is the recommended method of detecting BV.

Or

Acridine orange films or wet preparations may be used for the detection of clue cells, but are not as sensitive as the Gram film and are not recommended for optimal results[50,52]. If clue cells or other abnormalities (such as lack of lactobacilli and/or the presence of numerous small rods) are present, then a new smear should be made (preferably from the vaginal discharge), Gram stained, and examined applying Nugent or Hay criteria (see section 5.1).

Culture for TV

Perform on specimens with clinically suspected *T. vaginalis* or other sexually transmitted disease (see 4.3.1), and all pregnant women. Routine screening may be justified in areas of higher prevalence.

Microscopy for TV

Acridine orange films or wet preparations may be used for the detection of TV when routine screening is required. However, these methods are less sensitive than culture and are not recommended for optimal results[14,15].

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

Products of conception

Grind or homogenise specimen with a sterile tissue grinder (Griffiths tube or unbreakable alternative), sterile scissors and petri dish, or a pestle and mortar. The addition of a small amount of sterile, filtered water, saline, peptone or broth will aid the homogenisation process.

All grinding or homogenisation must be performed in a microbiological safety cabinet.

Aspirates, fluids

If sufficient sample is received, centrifuge at 1500 x g for 10min.

Decant the supernatant leaving approximately 0.5mL.

Resuspend the deposit in the remaining fluid.

4.3.2 Specimen processing

Culture for TV

Perform on specimens with clinically suspected *T. vaginalis,* or other sexually transmitted infection.

Method 1

Place the swab into a bijou bottle containing *Trichomonas* culture medium. This should be performed after inoculation of culture plates unless a separate swab is sent, because the medium contains antimicrobial agents.

Incubate in air at 35-37°C for 40-48hr.

Do not mix the culture after incubation. Withdraw some of the deposit from the bottom of the bottle with a pipette.

Place a drop on a clean microscope slide and over-lay with a coverslip.

Examine for the presence of motile trichomonads with a low power objective.

Method 2[13]

Pipette 100µl of *Trichomonas* culture medium to each well of a 96-well flat-bottomed microtitre tray.

Carefully swirl the genital swab in the appropriately labelled well.

Add a further 200µl culture medium to each well, cover with clear microplate sealer and incubate in air at 35-37°C for 40-48hr.

Examine at 16h-48hr, without removing the seal, for the presence of motile trichomonads with an inverted microscope under the low power objective.

Note: Do not remove microplate seal after application as cross contamination of wells may occur. Because of this, it is advisable to perform culture by this method as a batch towards the end of the working day. Microscopy should be performed through the seal.

4.4 Microscopy

4.4.1 Standard

Note: A direct, thin smear from the patient's exudate/discharge is the preferred specimen.

Note: Smears made from swabs in charcoal transport medium are not ideal for examination of specimens where gonorrhoea is suspected.

Microscopy for BV

Vaginal swabs.

Vaginal swabs from females of childbearing age with a diagnosis of vaginal discharge.

Either

Perform Gram stain and apply Nugent or Hay criteria (see section 5.1)[101].

Or

Acridine orange stained smear or wet preparation for clue cells with a Gram stained smear to confirm the presence of clue cells.

Microscopy for gonorrhoea

Cervical, endocervical, and female urethral smears and all male urethral specimens from suspected *N. gonorrhoeae* or known *N. gonorrhoeae* contact (unless previously performed in GUM clinic).

Prepare a thin smear on a clean microscope slide for Gram staining.

Aspirates, fluids and pus (or swabs of these)

Using a sterile pipette place one drop of the centrifuged deposit (see section 4.5), or neat specimen if there is insufficient to centrifuge, on a clean microscope slide.

Spread this with a sterile loop to make a thin smear for Gram staining.

Screening swabs for *N. gonorrhoeae.*

Microscopy may be performed in the GUM clinic.

IUCDs

Rub the surface of the IUCD thoroughly with a sterile swab, previously moistened with sterile water or saline. After inoculation of all agar plates, prepare a thin smear on a clean microscope slide for Gram staining. If any pus or exudate is present prepare the smear from this.

4.4.2 Supplementary

Wet preparation for the detection of TV

After inoculation of all agar plates, prepare a wet prep by rotating the swab (or placing a drop of vaginal discharge) on a clean microscope slide.

Place a coverslip over the wet inoculum and examine with a low power objective.

Acridine orange film for the detection of TV

After inoculation of all agar plates, prepare a thin smear on a clean microscope slide for acridine orange staining.

Note: Methods for staining procedures are contained in separate SMIs.

4.5 Culture and Investigation

Swabs

Inoculate each agar plate with swab (see Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Aspirates, fluids

With a sterile pipette, inoculate each agar plate with centrifuged deposit (see section 4.3.1 or neat specimen see Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

IUCDs

Rub the surface of the IUCD thoroughly with a sterile swab. Inoculate each agar plate with the swab (see Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Products of conception

Using a sterile pipette inoculate each agar plate with homogenised specimen (see Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
All HVS	Blood agar*	35-37	5-10% CO_2	16-24hr	16-24hr	*S. aureus* Lancefield Groups A, C and G streptococci Other organisms may be significant (see 4.6.1) eg Lancefield group B streptococci in pregnancy
	Sabouraud agar	35-37	air	40-48hr†	≥40hr	Yeasts
Urethral swabs Cervical swabs	Blood agar*	35-37	5-10% CO_2	16-24hr	16-24hr	*S. aureus* Lancefield Groups A, C and G streptococci Other organisms may be significant (see 4.6.1)
	Sabouraud agar	35-37	air	40-48hr	≥40hr	Yeasts

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	GC selective agar with antifungal agent	35-37	5-10% CO_2	40-48hr	≥40hr	*N. gonorrhoeae*

Note: If a vaginal swab is received in combination with a cervical and urethral swab, include standard media only with the vaginal and urethral swabs and add supplementary media as appropriate for the cervical swab.

4.5.1 Culture media, conditions and organisms (continued)

For these situations add the following:

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp° C	Atmos	Time		
Clinically suspected TV STD Pregnancy	*Trichomonas* medium	35-37	air	40-48hr	≥40hr	*T. vaginalis*
Intra-uterine death Septic abortion Miscarriage Balanitis Balanoposthitis Epididymitis† Orchitis	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
	CLED agar	35-37	air	≥16hr	≥16hr	Enterobacteriaceae Pseudomonads
?Listeriosis Intra-uterine death Septic abortion Miscarriage	*Listeria* selective agar	35-37	air	40-48hr	daily	*Listeria*
<10 years old	Chocolate agar	35-37	5-10% CO_2	40-48hr	daily	*H. influenzae*
?Actinomyces (clinically indicated or suggested by microscopy)	Blood agar supplemented with metronidazole and nalidixic acid	35-37	anaerobic	10d	≥40hr, at 7d and 10d	*Actinomyces*
?chancroid‡	*H. ducreyi* selective agar	33-34	5-10% CO_2	5d	5d	*H. ducreyi*

Other organisms for consideration - *T. vaginalis, C. trachomatis, Mycoplasma* species and viruses.

*incubation may be extended to five days; in such cases plates should be read at ≥40hr and left in the incubator/cabinet until day five.

†urine specimens may be investigated for these conditions (see B 41 - Investigation of Urine).

‡often a clinical diagnosis - refer to local protocols.

4.5.1 Culture media, conditions and organisms (continued)

All STI screening swabs

For all specimens:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	**Atmos**	**Time**		
?STI	GC selective agar with antifungal agent	35-37	5-10% CO_2	40-48hr	≥40hr	*N. gonorrhoeae*
For these situations add the following:						
Clinical details/ conditions	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
?STI (if required by local protocol)	Sabouraud agar	35-37	air	40-48hr	≥40hr	Yeasts

Other organisms for consideration: *T. vaginalis, C. trachomatis, Mycoplasma species, T. pallidum* and viruses.

4.5.1 Culture media, conditions and organisms (continued)

Aspirates/pus and swabs from tubo-ovarian abscess (TOA), fallopian tube, Pouch of Douglas (PoD), Bartholin's gland, IUCD and surgical specimens. For all specimens:

Clinical details/ conditions	**Standard media**	**Incubation**			**Cultures read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
PID Salpingitis TOA	Chocolate agar	35-37	5-10% CO_2	40-48hr	daily	*H. influenzae*
Bartholin's abscess Pyosalpinx Products of conception	Blood agar	35-37	5-10% CO_2	40-48hr	daily	*S. aureus* Streptococci Enterobacteriaceae
Infected IUCD Other inflammatory conditions	Fastidious anaerobe agar	35-37	anaerobic	5d	≥40hr and at 5d	Anaerobes
	GC selective agar with antifungal agent	35-37	5-10% CO_2	40-48hr	daily	*N. gonorrhoeae*
For these situations add the following:						
Clinical details/ conditions	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
? Actinomyces (clinically or suggested by microscopy)	Blood agar supplemented with metronidazole and nalidixic acid	35-37	anaerobic	10d	≥40hr, at 7d and 10d	*Actinomyces*
If microscopy suggestive of mixed infection	Neomycin fastidious anaerobe agar with metronidazole 5mg disc	35-37	anaerobic	5d	≥40hr and at 5d	Anaerobes
	CLED agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae
Optional media		**Incubation**			**Cultures read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
Either: Non-supplemented or supplemented blood culture bottles* or Supplemented brain heart infusion		35-37	air	continuous monitoring (minimum 40-48hr)	N/A	Any organism

broth Subcultured as appropriate at ≥40hr on to the standard media					
	35-37	air	40-48hr	daily	
	35-37	as above	as above	as above	

Note: a growth of any organism may be significant.

*follow manufacturer's recommendations.

Other organisms for consideration: *C. trachomatis*, *Mycoplasma* and *Ureaplasma* species, *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species) and viruses.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Actinomyces	"actinomycetes" level
Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Other streptococci and enterococci	genus level
Enterobacteriaceae	"coliforms" level
Haemophilus	species level
Listeria	species level
Neisseria	species level
Pseudomonads	"pseudomonads" level
S. aureus	species level
Yeasts	"yeasts" level

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

Gram films

Report on yeasts, WBCs, if present, and on the presence or absence of intracellular Gram negative diplococci.

Report on organisms seen in films from aspirates/pus (local reporting procedures should be followed on reporting of organisms seen in other specimens).

Report on clue cells if present and whether microscopy is suggestive of BV according to the criteria of Nugent (number of organisms per high power, oil immersion field (hpf) at approximately x1000 magnification) or of Hay[54]:

Nugent's criteria[54]

Numbers of *Lactobacillus* morphotypes seen	Score	Numbers of *Gardnerella* and *Prevotella* morphotypes seen	Score	Numbers of *Mobiluncus* morphotypes seen	Score
>30/hpf	0	>30/hpf	4	>30/hpf	2
5-30/hpf	1	5-30/hpf	3	5-30/hpf	2
2-4/hpf	2	2-4/hpf	2	2-4/hpf	1
1/hpf	3	1/hpf	1	1/hpf	1
none	4	none	0	none	0

Code each morphotype separately according to numbers of organisms seen as indicated in the table above and add individual scores together. Interpret scores as follows:

Total score 0-3	normal	
Total score 4-6	intermediate: suggestive of BV	Assess with clinical criteria and send repeat to confirm
Total score ≥7	abnormal: indicative of BV	

Hay's criteria[72]

Grade I	normal	predominantly *Lactobacillus* morphotypes
Grade II	intermediate	mixed *Lactobacillus* and other morphotypes. Assess with clinical criteria and send repeat to confirm if necessary
Grade III	abnormal	few or absent *Lactobacillus* morphotypes, but greatly increased number of *G. vaginalis* and other bacterial morphotypes. Suggestive of BV

Note: A vaginal smear should be requested on any swab that is suggestive of BV or if examination for BV is specifically requested.

Wet preparations or acridine orange films.

Report on WBCs, yeasts and trichomonads seen.

Note: A negative microscopy result does not exclude the possibility of TV infection.

5.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report: 16-72hr.

5.2 Culture

Report clinically significant organisms isolated **or**

Report other growth (eg normal flora isolated) **or**

Report absence of specific pathogens **or**

Report absence of growth.

The absence of *N. gonorrhoeae* in vaginal swabs should not be reported as these are not the specimen of choice for the isolation of *N. gonorrhoeae*. Recommendations on the appropriate specimen type should be included in the report.

Also, report results of supplementary investigations.

According to local protocols for reporting the carriage of Group B Streptococci, it may be appropriate for laboratories to report isolates of Group B streptococci to the Ante-natal Clinic.

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically.

Written report: 16-72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations: see appropriate SMIs.

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[102,103] or Equivalent in the Devolved Administrations[104-107]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[104,105], Wales[106] and Northern Ireland[107].

It may be appropriate for laboratories to report isolates of Group B streptococci to the Ante-natal Clinic as according to local protocols for the reporting of the carriage of these isolates.

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UK Standards for Microbiology Investigations

Investigation of specimens for screening for MRSA

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

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Bacteriology | B 29 | Issue no: 7 | Issue date: 26.05.20 | Page: 1 of 18

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Contents

Acknowledgments 2

Amendment table 4

1 General information 6

2 Scientific information 6

3 Scope of document 6

4 Background 6

5 Safety considerations 9

6 Diagnostic tests/investigation 9

7 Antimicrobial susceptibility testing 12

8 Referral to reference laboratories 13

Appendix: Investigation of specimens for screening for MRSA 14

References 15

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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	8/03.04.14
Issue no. discarded.	5.2
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated.
Introduction.	Introduction has been restructured to aid flow. Livestock MRSA has been inserted. Strength of enrichment broth recommended changed from 7% to 2.5%.
Appendix.	Old Appendix 1 deleted. Old Appendix 2 has become Appendix 1 with a link replacing the table. Old Appendix 3 has become Appendix 2.
References.	References reviewed and updated.

Amendment number/date	9/26.05.20
Issue number discarded	6
Insert issue number	7
Anticipated next review date*	26.05.23

Section(s) involved	Amendment
Whole document	Document written in new template Introduction, background information and references updated Information on sample collection added

*Reviews can be extended up to five years subject to resources available.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standard for Microbiology Investigation (UK SMI) describes the processing of screening human specimens to detect meticillin-resistant *Staphylococcus aureus* (MRSA).

Guidelines for the control of MRSA in healthcare facilities have been produced by a working party of the Healthcare Infection Society (HIS), the British Society for Antimicrobial Chemotherapy (BSAC) and the Infection Control Nurses Association (ICNA)[1]. These guidelines recommend a risk assessment approach and advise Infection Control Committees to adapt them locally when designing infection control policies. Other recommendations and epidemiological data have been published by the Scottish Infection Standards and Strategy Group (SISSG)[2], the Department of Health (DH)[3] and Public Health England (PHE).

This UK SMI should be used in conjunction with other UK SMIs.

4 Background

Meticillin was the first penicillinase resistant penicillin and has been widely used in testing susceptibility of *S. aureus* to penicillinase resistant β-lactam agents. Hence, despite the fact that meticillin is no longer available and oxacillin and cefoxitin have replaced it for susceptibility testing, resistant strains are commonly known as MRSA.

MRSA strains are a continuing problem in healthcare settings, with transmissions and outbreaks now occurring in the community. Screening for MRSA provides a means of identifying patients and staff who may be at risk of infection and/or involved in transmission of the organism.

In order to achieve the most effective use of finite hospital resources and to minimise morbidity due to these organisms it is usual to have a policy of planned screening to guide control measures to protect patients from MRSA colonisation and infection. Precisely what patient and staff screening is performed will depend on the endemicity of the problem and the case mix of the unit. If MRSA is highly endemic, with constant challenges to the provider units, then a risk assessment process is recommended. One approach is to concentrate on patients at greatest risk. Screening may also be appropriate in areas with low patient risk, particularly so where there is extensive interaction and transfer of patients with MRSA among wards or to acute care wards.

Infection risks

Studies have shown that the majority of patients from whom MRSA is isolated are colonised rather than infected with the organism[4]. Factors predisposing to colonisation

include procedures involving "hands on" care especially in acute surgical, renal dialysis and critical care units[5]. The risk of colonisation resulting in infection is increased in the presence of any breach in the skin, such as surgical wounds and devices penetrating the skin, for example prostheses and catheters. These breaches provide a portal of entry for bacteria[5]. Eradication of nasal carriage of *S. aureus* may be beneficial in certain clinical conditions such as recurrent furunculosis.

Mechanisms of resistance

Intrinsic resistance to β-lactams in clinical strains of *S. aureus* is often heterogeneous[6]. High-level resistance is expressed by a minority of cells on ordinary media at 37°C but more uniformly in hypertonic media or at 30°C[7,8]. Although most MRSA produce a β-lactamase, this is not responsible for their resistance to meticillin. Classical MRSA contain the *mecA* gene and this is the essential determinant of meticillin resistance. *MecA* is a 2,130 base pair segment of DNA coding for a penicillin-binding protein (PBP2' or PBP2a) characterised by a low affinity for most β-lactams, and which is thought to take over the functions of all other PBPs when they are saturated by meticillin or other β-lactam antibiotics. Methicillin-sensitive Staphylococcus aureus (MSSA) do not produce this protein and their DNA will not hybridise with a probe specific for the *mecA or mecC* gene. The genetic determinant of PBP2a and PBP2c is transcribed in all MRSA cells and all phenotypic classes of MRSA, but additional factors affect the expression of meticillin resistance.

The *mecA* gene is part of a mobile genetic element, the SCC*mec*, which is incorporated in the chromosome[9]. Twelve distinct types of SCC*mec*, designated I to XI have been described to date[10-13]. Most healthcare associated (HA-MRSA) harbour types I, II or III whereas most community-associated (CA-MRSA) harbour types IV or V, although EMRSA-15 encode type IV[14].

More recently, a *mec*A homologue which shows only 69% homology with *mec*A has been described. The gene is now known as *mec*C and is carried in a mobile element known as SCC*mec*XI which has been identified in MRSA from humans and animals including wildlife and livestock.

The presence of the *mecA* and *mecC* genes and oxacillin, meticillin or cefoxitin MIC above breakpoints recommended by national and international validated methods are accepted criteria for methicillin resistance.

Borderline resistance

Some strains of *Staphylococcus aureus* may be encountered which are *mecA* and *mecC* negative but which exhibit a borderline resistance. This may be due to hyperproduction of β-lactamase (particularly obvious when testing oxacillin susceptibility) or alteration of PBPs[15]. There is some evidence from animal models that hyperproduction of β-lactamase is not clinically significant, but further data on virulence and effectiveness of therapy of patients infected with borderline resistant strains are needed to determine whether control measures are warranted[16,17].

Multiple drug resistance

The most prevalent epidemic MRSA strains in the UK remain susceptible to several antibiotics including the glycopeptides vancomycin and teicoplanin. However, MRSA strains showing reduced susceptibility to vancomycin have been described[18]. This eventuality should be considered in any patient with MRSA in whom there is an

apparent treatment failure with a glycopeptide antibiotic[19]. Some strains now demonstrate resistance to as many as 20 antimicrobial compounds, including antiseptics and disinfectants and this trend in acquisition of extra resistances appears to be increasing[6]. Despite this there are several agents that are appropriate for the treatment of MRSA infections and new agents are being developed and introduced[19].

Antibiotic susceptibility

Detection of a presumptive MRSA strain should be followed by its full identification as *S. aureus,* confirmation of meticillin resistance and testing susceptibility to other antimicrobial agents. Conventional oxacillin susceptibility tests are markedly affected by test conditions and the use of cefoxitin in disc diffusion tests has been shown to be less affected by test conditions and to be more reliable than tests with oxacillin[20,21]. Both disc diffusion and breakpoint methods are widely used.

Recommended screening methods

Routine screening by direct plating:

A chromogenic selective MRSA agar has the benefit of providing a rapid result (preliminary results after overnight incubation) although is less sensitive than other methods.

Screening by molecular methods:

Use of a commercial method applied directly to screening swabs may be considered if very rapid results are required.

Screening by enrichment:

In particular circumstances (for example checking patients for clearance of MRSA) screening by an enrichment method may be used. Several swabs from different sites from the same patient can be combined in the same 2.5% NaCl nutrient broth. This is a cost-effective method where the aim is to determine the presence, rather than the site, of MRSA carriage.

Both direct plating and enrichment methods may be used. Enrichment delays reporting of results by 24hr but negative results with a more sensitive technique (enrichment) may be required before MRSA control measures are discontinued for the patient[22].

Screening by selective broth:

Culture of MRSA screening swabs in selective broth can increase the sensitivity of the test, provided that the selective medium is not inhibitory to the MRSA strain involved. A range of commercially available selective broth can be used. These generally contain cefoxitin which is principally aimed at inducing the expression of meticillin resistance and inhibiting the growth of MSSA[23].

5 Safety considerations

Containment Level 2.

Refer to current guidance on the safe handling of all organisms documented in the safety considerations section of the UK SMI scientific information (see section 2).

The above guidance should be supplemented with local COSHH and risk assessments."

6 Diagnostic tests/investigation

6.1 MRSA laboratory screening

6.1.1 Specimen type

Recommended MRSA screening specimens (a combination of three swabs from different body sites): nose, throat, axilla, groin (or perineum) and rectum[24].

In addition, if a patient has a long-term catheter a catheter urine specimen must be taken.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage[25,26,27]

Unless otherwise stated, swabs for MRSA culture should be placed in appropriate transport medium[28-32].

Self-collected samples should follow manufacturer recommendations.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Specimens for molecular methods should follow manufacturer's instructions.

Specimens should be transported and processed as soon as possible[27].

Swabs may be placed directly in enrichment broth on the ward. Swabs in enrichment broths should not be refrigerated. If ward staff are involved they should be adequately trained.

Swabbing

Use of correct swabbing technique has been shown to improve bacterial recovery rate[33]. Pooled swabs may be used.

Procedure

Swabs should be taken from the following sites:

- nose: carefully place swab into nasal vestibule (not the middle nor back of the nose) and rotate around 5 times (5 seconds). One swab can be used for both nostrils
- groin: in the fold between the perineum and the thigh, swab should be rolled around several times

- any skin lesions / PEG (percutaneous endoscopic gastrostomy) sites / catheter sites/ drain sites
- consider the throat, especially in suspicion of prolonged carriage

A combination of 3 sites (nose plus another 2 sites: axilla, throat, groin, perineum or rectum) should be used[24].

Note: if the patient is catheterised a catheter urine specimen should be taken

6.1.3 Laboratory processes (analytical stage)

Culture

Ideally, a screening method should allow the growth of all MRSA, inhibit or differentiate other organisms, and allow direct identification tests to be performed on colonies. Unfortunately, some of these requirements conflict and a compromise is necessary.

Conventional methods used for screening should detect strains of MRSA by inhibiting contaminants and selecting *S. aureus* strains which are meticillin resistant. Direct plating on selective medium has the advantage that results may be available within 24hr, but most studies indicate that direct plating is less sensitive than broth enrichment followed by plating on solid media[34]. Whether this is the case with more recently developed chromogenic media remains to be determined. Sodium chloride, antibiotics and other selective agents may be added to the media to reduce contamination, although they might inhibit *S aureus* and the other antibiotics such as oxacillin or cefoxitin that are normally added to select methicillin resistant strains[35,36].

Enrichment broth containing 7% NaCl may inhibit the growth of some isolates of MRSA if present in small numbers[37]. For this reason 2.5% NaCl is recommended as it has been shown to work well when sub culturing to chromogenic agar[38].

Mannitol Salt Agar (MSA) and variations of MSA have been widely used but have the disadvantage that direct agglutination tests for identification of *S. aureus* on MSA are unreliable or growth of MRSA is slow. The HIS/BSAC/ICNA working party and other reports consistently show chromogenic media to perform well although some require a longer incubation period than others and confirmation from this media using latex agglutination cannot be relied upon[34,39,40].

Specimen processing

Direct culture

Inoculate each agar plate with swab or other sample (Q 5 – Inoculation of culture media for bacteriology).

Enrichment culture

Remove the cap aseptically from the container and place the swab(s) or other specimen in the broth, break off (or cut) the swab-stick(s) and replace the cap.

Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Direct culture	MRSA screening specimens	Chromogenic selective MRSA medium	35-37	Aerobic	18-48hr**	daily	MRSA
OR							
Enrichment culture		Nutrient broth containing 2.5% NaCl *** then subculture to (*see below*)	30	Aerobic	18-24hr	N/A	
		Chromogenic selective MRSA medium	35-37	Aerobic	18-48hr**	daily	MRSA

* Molecular methods may be considered if a rapid result is required.

**For chromogenic media refer to manufacturer's instructions for recommended incubation times.

***The bottle should contain a volume of broth sufficient to cover the swabs. The NaCl concentration should be reduced if locally prevalent strains are known to be inhibited by 2.5% NaCl.

Identification

Minimum level of identification in the laboratory

S. aureus species level, cefoxitin resistant.

Organisms may be further identified if this is clinically or epidemiologically indicated.

Molecular methods

Molecular methods for detection of MRSA require target specific detection of *Staphylococcus aureus* (via the *nuc*, gyrB, or the *Staphylococcus* protein A gene) together with identification of meticillin resistance (via SCC*mec*-orfX, fem A, or *mec*A). Different commercial kits use different combinations of these targets. However, the emergence of novel *mec* variants (eg *mec*C) means that targets for detection of meticillin resistance need continuous re-evaluation[34,41]. Assessment of available methods indicated good performance and results in 2-3hr even using in house methods[42]. Variations in the conserved regions of the SCCmec elements need to be monitored as some commercial kits fail to detect MRSA when there are nucleotide polymorphisms in this area.

Other methods used in MRSA screening

Other methods giving more rapid results may be considered, such as the latex agglutination-based method that detects the PBP2a protein which is commercially available[43]. Although consideration to local prevalence rates of MRSA needs to be considered when using them[44].

6.1.4 Post-laboratory processes (reporting procedures)

Culture

Interpreting and reporting results

Negatives

"MRSA not isolated"

Positives

"MRSA isolated"

Reporting time

Clinically urgent culture results to be telephoned or sent electronically when available.

Presumptive positive result should be reported (subject to local policy)

7 Antimicrobial susceptibility testing

Refer to EUCAST guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

This UK SMI recommends selective and restrictive reporting of susceptibilities to antimicrobials. Any deviation must be subject to consultation that should include local antimicrobial stewardship groups.

It is recommended that the antimicrobials in bold in the table below are reported. Those antimicrobials not in bold may be reported based on local decisions.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
MRSA	**Oxacillin** **Flucloxacillin** Clarithromycin Clindamycin **Vancomycin** **Mupirocin***	Daptomycin Linezolid Trimethoprim Rifampicin Tigecycline Ceftaroline Teicoplanin Fucidic acid	* No breakpoint data provided by EUCAST

7.1 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated.

MRSA should not be reported as susceptible to any currently available β-lactams although there are new β-lactam agents that are being introduced that have some activity against MRSA[45].

8 Referral to reference laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory see GOV.UK for user manuals and request forms.

The national Staphylococcus Reference Service in Public Health England (PHE) invites the referral of *S. aureus* strains showing unusual resistance (specifically to vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin, daptomycin, tigecycline, ceftaroline or ceftibiprole) for analysis and surveillance purposes.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

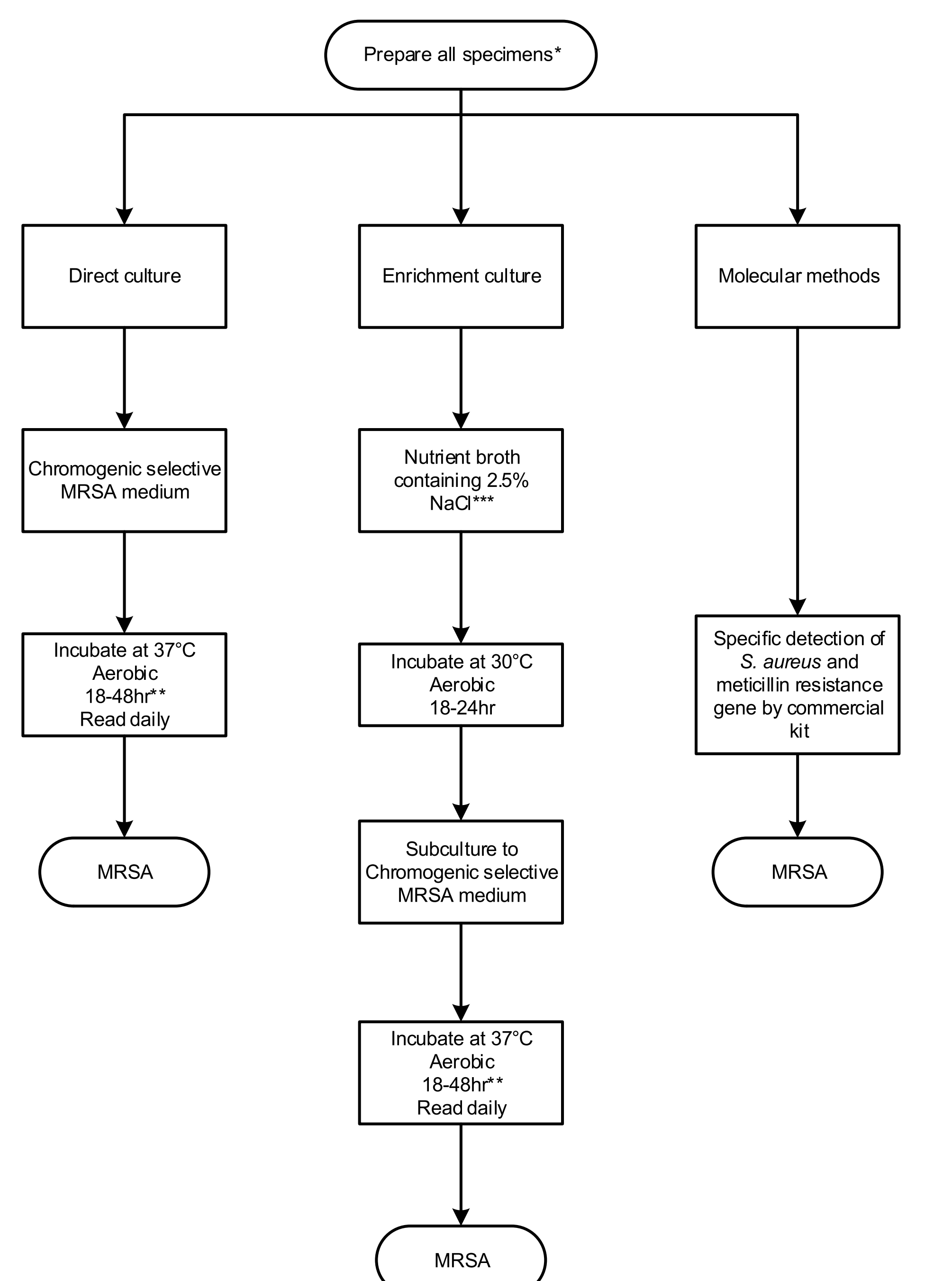
Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Appendix: Investigation of specimens for screening for MRSA



* Consider a molecular method if rapid results are required
** For chromogenic media refer to manufacturer's instructions for recommended incubation times
*** The bottle should contain a volume of broth sufficient to cover the swabs.

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For the information for the evidence grade ratings given, refer to the scientific information link above in section 2.

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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 29 Investigation of specimens for screening for MRSA

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

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Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

Consultation: 19/01/2018 – 02/02/2018

Version of document consulted on: B 29de+

Proposal for changes

Comment number	1		
Date received	19/01/2018	**Laboratory/Professional body**	Laboratory
Section	Pages 11-13		
Comment			
Only one minor comment: On page 11 it states that: 'Direct plating on selective medium has the advantage that results may be available within 24hr, but most studies indicate that direct plating is less sensitive than broth enrichment followed by plating on solid media. Whether this is the case with more recently developed chromogenic media remains to be determined'. Also, on page 13 it states that 'The advantage of enrichment over direct plating has yet to be confirmed with chromogenic media.' In my view there are now a number of studies that show that enrichment culture will increase the yield of MRSA whether chromogenic media are used or not. Three examples of such studies are provided below. Some studies that show conflicting results have often used quite inhibitory broths (e.g. containing 7.5% salt). Dodémont M, Verhulst C, Nonhoff C, Nagant C, Denis O, Kluytmans J. Prospective Two-Center Comparison of Three Chromogenic Agars for Methicillin-Resistant Staphylococcus aureus Screening in Hospitalized Patients. J Clin Microbiol. 2015 Sep;53(9):3014-6. https://www.ncbi.nlm.nih.gov/pubmed/26109446 Veenemans J, Verhulst C, Punselie R, van Keulen PH, Kluytmans JA. Evaluation of brilliance MRSA 2 agar for detection of methicillin-resistant Staphylococcus aureus in clinical samples. J Clin Microbiol. 2013 Mar;51(3):1026-7. doi: 10.1128/JCM.02995-12. http://jcm.asm.org/content/51/3/1026.full Wolk DM, Marx JL, Dominguez L, Driscoll D, Schifman RB. Comparison of MRSASelect Agar, CHROMagar Methicillin-Resistant Staphylococcus aureus (MRSA) Medium, and Xpert MRSA PCR for detection of MRSA in Nares: diagnostic accuracy for surveillance samples with various bacterial densities. J Clin Microbiol. 2009 Dec;47(12):3933-6. https://www.ncbi.nlm.nih.gov/pubmed/19828738			
Evidence			
Provided above.			
Financial barriers			
No.			
Health benefits			

No.	
Recommended action	PARTIAL ACCEPT: Group advised that the use of direct culture on chromogenic agar should always be recommended over enrichment although enrichment is more sensitive. Sentence "The advantage of enrichment over direct plating with chromogenic media has yet to be confirmed" has been removed to avoid confusion. References included in document

Comment number	2		
Date received	23/01/2018	**Laboratory/Professional body**	Laboratory
Section	Introduction and Technical information/limitations		
Comment			
Typo on page 9 transfers and Under technical information/limitations the draft SMI states that Staphylococcus sciuri can ...grow on chromogenic MRSA medium with a blue green pigment. This statement assumes that all MRSA chromogenic media produce a blue chromogen but this is not the case (see links in evidence below).			
Evidence			
http://www.chromagar.com/clinical-microbiology-chromagar-mrsa-focus-on-mrsa-28.html#.WmdzvI-0Pmg http://www.biomerieux-diagnostics.com/chromid-mrsa-smart http://hardydiagnostics.com/chromogenic-mrsa-staphylococcus-aureus-mrsa-identification-by-chromogenic-media-hardychrom-mrsa/			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	ACCEPT Note: section was removed from document as introduction was reduced		

Comment number	3		
Date received	31/01/2018	**Laboratory/Professional body**	Laboratory
Section			

Comment
1. "MRSA strains are a continuing and increasing problem in healthcare settings…" Not sure what evidence there is for this in the UK currently, esp with MRSA bacteraemia rates declining steeply 2. "MRSA and MSSA are similar in virulence and this is often connected to mobile genetic elements the presence or absence of which determines the clinical outcome" this is in odds with more recent literature – e.g. see J Infect Dis. 2012 Mar 1;205(5):798-806. doi: 10.1093/infdis/jir845 and The ISME Journal (2010) 4, 577–584; doi:10.1038/ismej.2009.151; 3. In mechanisms of resistance: add mecC and PBP2c where corresponds. 4. "Eleven distinct types of SCCmec" this should be twelve (doi:10.1128/AAC.01692-15) 5. Section 5.3 – antimicrobial susceptibility testing: add the following: "The national Staphylococcus Reference Service in Public Health England (PHE) invites the referral of S. aureus strains showing unusual resistance (specifically to vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin, daptomycin, tigecycline, ceftaroline or ceftibiprole) for analysis and surveillance purposes."
Evidence
Not completed.
Financial barriers
Not completed.
Health benefits
Not completed.

Recommended action	
	1. ACCEPT: sentence changed by removing the word increasing. 2. ACCEPT: sentence has been removed as group estimated it creates more confusion and adds nothing to the whole paragraph. 3. ACCEPT: mecC and PBP2c added 4. ACCEPT: changed to twelve and reference added 5. ACCEPT: suggested sentence added Other minor changes to text where accepted from the noted and reviewed version.

Comment number	4		
Date received	02/02/2018	**Laboratory/Professional body**	Laboratory

Section	page 9
Comment	
Whole section feels very out-dated, especially the paragraph wi:th references 13 and 14 which date back to the 1990s. Needs a complete refresh, taking into account current data on the balance between MRSA and MSSA.	
Evidence	
Not completed.	
Financial barriers	
Not completed.	
Health benefits	
Not completed.	
Recommended action	Whole section removed from introduction in new document/template

Comment number	5		
Date received	02/02/2018	**Laboratory/Professional body**	Laboratory
Section	2 & 4		
Comment			
1. 2.2 Remove reference to fungal culture as this is MRSA screening so not relevant for this document. 2. 2.3 Are there any guidelines for what are the minimum sites to be tested or is this a local agreement only? Swabs from nose, axilla, groin, etc. and urine specimens are appropriate specimens. 3. 4.5.1 Temperature for incubation should be in a range, e.g. 35-37 degrees C and not a fixed 37 degrees C. A fixed temperature is unattainable from a UKAS standard perspective. 4. 4.7 Should BSAC guideline be removed as this is no longer the recommended method.			
Evidence			
Not completed.			
Financial barriers			
Not completed.			
Health benefits			
Not completed.			

Recommended action	1. ACCEPT: sentence replaced by: "Unless otherwise stated, swabs for MRSA culture should be placed in appropriate transport medium" 2. ACCEPT: a new section describing swabbing sites and procedure has been added to the document. 3. ACCEPT: temperature modified to show range and not fixed temperature 4. ACCEPT: BSAC guidelines removed

Comment number	6		
Date received	02/02/2018	**Laboratory/Professional body**	Professional body
Section	Mechanisms of resistance		
Comment			
Paragraph 4 doesn't make grammatical sense. Perhaps the sentence could be replaced with: The presence of the mecA gene or proven resistance to oxacillin, meticillin or cefoxitin, using methodologies recommended by EUCAST (BSAC) or NCCLS, are accepted criteria for confirmation of meticillin resistance.			
Evidence			
Not completed.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	PARTIAL ACCEPT: sentence replaced for clarity: "The presence of the mecA and mecC genes and oxacillin, meticillin or cefoxitin MIC above breakpoints recommended by national and international validated methods are accepted criteria for methicillin resistance."		

Respondents indicating they were happy with the contents of the document

Overall number of comments: 3			
Date received	22/01/2018	**Laboratory/Professional body**	Professional body
Health benefits			
No.			

Date received	25/01/2018	**Laboratory/Professional body**	Professional body
Health benefits			
Not completed.			
Date received	30/01/2018	**Laboratory/Professional body**	Commercial company
Health benefits			
Yes.			

UK Standards for Microbiology Investigations

Investigation of specimens other than blood for parasites

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 31 | Issue no: 5.1 | Issue date: 28.06.17 | Page: 1 of 60

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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PHE publications gateway number: 2016309

UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

Acknowledgments **2**

Amendment table **5**

UK SMI: scope and purpose **6**

Scope of document **9**

Introduction **9**

Carriage rate **9**

Technical information/limitations **24**

1 Safety considerations **26**

2 Specimen collection **27**

3 Specimen transport, storage and retention **29**

4 Specimen processing/procedure **30**

5 Reporting procedure **36**

6 Notification to PHE, or equivalent in the devolved administrations **37**

Appendix 1: Specimen types and possible parasites detectable **38**

Appendix 2: Geographic distribution of parasitic infections **40**

Appendix 3: Calibrating the microscope for measurement **42**

Appendix 4: Common microscopic constituents of faeces **43**

Appendix 5: Relative sizes of helminth eggs **44**

Appendix 6: Oocysts of coccidia/ cryptogregaria/ microsporidia **45**

Appendix 7: Comparison of tapeworms commonly found in humans **46**

Appendix 8: Helminth larvae – characteristics **47**

Appendix 9: Identification of amoebic trophozoites in stained smears **48**

Appendix 10: *Balantidium coli* - trophozoite and cyst **49**

Appendix 11: Identification of amoebic and flagellate cysts **50**

Appendix 12: Identification of flagellate trophozoites **51**

Appendix 13: Microfilariae found in humans **52**

References **53**

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	10/28.06.17
Issue number discarded	5
Insert issue number	5.1
Anticipated next review date*	01.03.20
Section(s) involved	**Amendment**
Page 28.	Minor textual additions.
Page 33.	Minor clarity made to urine specimens for *S. haematobium.*

Amendment number/date	9/01.03.17
Issue number discarded	4.1
Insert issue number	5
Anticipated next review date*	01.03.20
Section(s) involved	**Amendment**
Whole document.	Updated the scope of the document to include the current molecular and traditional methods used for detection of parasites. Updated Technical Limitations section. Updated Safety Considerations section. References added. More diagrams added in the appendices.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

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Scope of document

Type of specimen

Faeces, urine, sellotape slide/perianal swab, CSF, bile, pus from abscesses, duodenal/jejunal aspirates, tissues, hydatid cyst, sputum/ bronchoalveolar lavage, biopsies from colonoscopy or surgery

This UK SMI describes the detection and isolation of a range of parasites (protozoa, nematodes, trematodes, cestodes) and organisms of previously uncertain taxonomic status traditionally included with the protozoa from a variety of clinical samples, excluding blood and corneal tissue scrapings. For corneal tissue scrapings for *Acanthamoeba* detection, refer to B 2 – Investigation of bacterial eye infections. This document covers the traditional and molecular methods of detection in detail.

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

Although faecal specimens are the most commonly received samples for the detection of parasites, parasites may also be detected in a variety of other clinical specimens submitted to the laboratory. Clinical presentation, specimen preparation and organism identification of the parasites most commonly seen in the laboratories are described here, but others may be found. For completeness, uncommon species are also described.

Reference laboratories should be used to identify those parasites outside the laboratory's normal experience. Haematology, histopathology, and serology laboratories may also contribute significantly to the laboratory diagnosis of parasitic infections.

This introduction covers:

- protozoa
    - amoebae
    - flagellates/ciliates
    - coccidia
    - cryptogregaria
- nematodes
- trematodes
- cestodes
- other organisms of previously uncertain taxonomic status

Carriage rate

The carriage rate will vary among parasites and this will largely depend on:

- infecting species of the parasite
- geographic distribution

- seasons
- migration and travelling
- residence
- age
- poor hygiene
- previous exposure
- predisposing susceptibility such as immunocompromise (for example, AIDS, malnutrition)

Protozoa[2]

Intestinal amoebae

Amoebae that may be isolated from the human gastrointestinal tract include *Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba coli, Entamoeba hartmanni, Endolimax nana* and *Iodamoeba butschlii*. All except *E. histolytica* are usually non-pathogenic (see Appendices 9-11).

E. histolytica and *E. dispar* are morphologically indistinguishable by light microscopy[3]. Of the two species, only *E. histolytica* is capable of causing invasive disease. When the diagnosis is made by light microscopy, cysts should be reported as *E. histolytica/E. dispar*[4]. Alternatively, diagnostic methods including antigen detection by enzyme immunoassay or DNA detection by PCR could be performed by laboratories to distinguish these two species[2,3,5]. Where such is not possible, it is advised that laboratories should forward the samples to the appropriate specialist centres for further confirmation.

E. histolytica may cause ulcerative and inflammatory lesions in the colon. It spreads to extraintestinal sites, most commonly the liver, where marked tissue destruction occurs, leading to abscess formation. Inflammation of the colon produces symptoms of dysentery which include lower abdominal pain, increased frequency of bowel movements and liquid stools. Infection can lead to perforation of the colon, toxic megacolon, amoeboma, and perianal ulceration[4].

Appropriate specimens include liquid aspirated or scraped from an area of inflamed bowel tissue or rectal scrape in dysentery, and freshly passed faeces for wet microscopy and formol-ether / ethyl acetate concentration. Motile trophozoites with ingested red blood cells found in stools, or trophozoites in tissue biopsies, both strongly suggest infection with *E. histolytica*[2].

Free-living amoebae

Human infection with free-living amoebae is uncommon. Such protozoa include: *Acanthamoeba* species, *Naegleria fowleri* , *Balamuthia mandrillaris* and *Sappinia diploidea*[6]. Infections include central nervous system invasion, mostly in immunocompromised individuals and acanthamoeba keratitis, common in contact-lens wearers.

Primary amoebic meningoencephalitis (PAM) is caused by *N. fowleri*. It occurs in adults and children who have recently been swimming in contaminated warm fresh water. Organisms gain access to the central nervous system by direct invasion

through the nasal mucosae. Although treatment is available and there are a handful; of reported survivors, the disease is usually fatal[6].

When patients present with purulent meningitis or meningoencephalitis and a history of recent exposure to fresh water, PAM should be considered. The peripheral white blood cell count may be low early on in the disease, but will increase in time. The cerebrospinal fluid (CSF) appearance is haemorrhagic, glucose is low or normal and protein is elevated.

Laboratory diagnosis may be made by examining a wet mount and a stained preparation of the CSF for amoebic trophozoites (Appendix 9 and 11).

Granulomatous amoebic encephalitis (GAE) is caused by *Acanthamoeba* species and *Balamuthia mandrillaris*. It is a chronic opportunistic infection, occurring more often in immunosuppressed/AIDS patients spreading haematogenously from pulmonary or skin lesions to the central nervous system and it is often fatal. Acanthamoeba keratitis is associated with the use of soft contact lenses and ocular trauma; if not treated promptly may lead to corneal ulceration, and eventually to blindness. However, *Acanthamoeba* species may be isolated from contact lens fluid from individuals with no signs or symptoms of disease (refer to B 2 – Investigation of bacterial eye infections)[6].

GAE may be diagnosed by examining brain biopsy material. Acanthamoeba infection of the skin is frequently present with GAE: skin nodules or ulcers may be biopsied and examined by wet microscopy and a stained preparation (Appendix 9). Serological methods are also available.

Flagellates

Giardia intestinalis (synonymous with *Giardia duodenalis and Giardia lamblia*)[7]

This organism may cause waterborne outbreaks of diarrhoea and is primarily spread from person to person, or zoonotically, via the faecal-oral route[2]. Infection may present as self-limiting diarrhoea or a syndrome of chronic diarrhoea, steatorrhoea, malabsorption and weight loss. Symptoms include diarrhoea, abdominal cramps, bloating and flatulence. Vomiting, fever and tenesmus can also occur, but infection may also be asymptomatic[2].

Laboratory diagnosis for faeces is made by microscopy, or by antigen detection using enzyme immunoassay (EIA) or immunochromatographic lateral flow (ICLF) assay, or by PCR. Borderline positive and questionable negative reactions obtained by EIA and ICLF should be confirmed by another method[7]. Microscopy is of low sensitivity (31%) compared to PCR and evidence suggests a doubling of detection rates using automated EIA[8,9]. One small study reported false positives by EIA compared to PCR[10]. The relative sensitivity of methods may be dependent on sample preparation and the assay used[11].

Due to the variable shedding of organisms, several stool specimens should be examined especially if microscopy is used. Ideally a total of three specimens should be taken 2-3 days apart.

Diagnosis can also be made by microscopic examination of duodenal or jejunal aspirates, and biopsies (see Appendix 11). Motile trophozoites can be seen in the direct examination of fresh faeces, duodenal and jejunal aspirates while cysts can be seen in a direct saline preparation or a formol-ether /ethyl acetate concentration of faeces.

Note: It should be noted it is not possible to identify trophozoites or cysts to species level by light microscopy. Molecular tests are required and are not currently available nationally.

Serology is not helpful and it is no longer available in the UK.

Dientamoeba fragilis[2]

The pathogenicity of *Dientamoeba fragilis* is debatable; however it has been documented in cases of non-invasive diarrhoea with fatigue. Unlike other protozoa it does not have a cystic stage, and is now regarded as amoeba/flagellate. The role of Dientamoeba in patients with HIV and bowel disorders is unclear and more research is required[12].

The trophozoite stage is very difficult to detect in wet saline preparations, and will not be seen in formol-ether/ethyl acetate concentrations. They can be detected by staining the faecal sample with trichrome, Giemsa or Field stains (TP 39 - Staining procedures). Alternative methods including DNA detection by PCR have been developed[12].

Trichomonas vaginalis and *Pentatrichomonas hominis*

Both *Trichomonas vaginalis* and *Pentatrichomonas hominis* may be found in human infections. The majority of infections caused by *T. vaginalis* are sexually transmitted and it affects mainly the genitourinary tract whereas *P. hominis* inhabits the large bowel and is usually regarded as a non-pathogen, although it may cause mild gastro-intestinal symptoms when present in large numbers (B 28 – Investigation of genital tract and associated specimens)[13].

Laboratory diagnosis is usually made by observing the motile trophozoites in the microscopic examination of a wet saline preparation. Preparations with stained acridine-orange may also be used, although this will involve fluorescence microscopy and consequential loss of immediacy. DNA detection by PCR has been developed for *Trichomonas vaginalis*[14,15].

Ciliates

Balantidium coli

B. coli is a ciliate infecting numerous mammals including humans and pigs and is found worldwide. Humans are usually resistant to *B. coli* infection, but achlorhydria or poor nutrition may increase the risk of colonisation. Colonisation is often asymptomatic. Patients may develop intermittent watery diarrhoea or an acute dysenteric colitis with stools containing mucus and blood[2].

Rapidly motile, large trophozoites may be observed microscopically in fresh faeces (Appendix 10). In preserved samples, diagnosis is by microscopy of a wet preparation, as neither the trophozoites or the cysts stain clearly with iodine or permanent stains[1].

Coccidia

Cyclospora

Cyclospora cayetanensis infection occurs in many tropical countries and outbreaks have been associated with drinking contaminated water and eating contaminated food affecting travellers and foreign residents[16-18]. Foodborne outbreaks have been reported in North America and Europe associated with the consumption of imported soft fruit, salad leaves, fresh herbs and vegetables[19,20].

Symptoms of *Cyclospora* infection include watery diarrhoea with weight loss, severe fatigue, nausea, vomiting and abdominal pain. Infection with *Cyclospora cayetanesis* also occurs in HIV-infected patients[21].

Laboratory diagnosis is by appearance of oocysts in wet preparations and concentration methods can be used. Oocysts give a characteristic blue autofluorescence at 340-360nm. Modified Ziehl-Neelsen may be used as a permanent stain. *Cyclospora cayetanesis* stains poorly with auramine-phenol stain (see TP 39 - Staining procedures and Appendices 3 and 6). Confirmation by microscopy and by PCR is available at the reference laboratories. There is no standardised subtyping scheme for *Cyclospora cayetanensis*.

Cystoisospora belli (formerly *Isospora belli*)[2]

Cystoisospora belli infection is relatively uncommon in developed countries, but is endemic in some parts of the world. It is usually transmitted by the ingestion of contaminated food or water. In immunocompetent patients it may cause non-specific, self-limited watery diarrhoea with malaise, anorexia, abdominal cramps and weight loss whereas severe infection can occur in immunocompromised hosts causing severe diarrhoea with electrolyte disturbances and morbidity[22]. AIDS patients are particularly susceptible to *Cystoisospora belli* infection (this being particularly common in underdeveloped countries especially Africa and the Middle East where the incidence ranges from 0.2% to 20% in patients with AIDS[23].

Laboratory diagnosis is achieved by microscopy of stool samples or intestinal mucus. Formol-ether concentration of faeces is recommended; as with *Cyclospora* species, *Cystoisospora* autofluoresce at 340-360nm (see TP 39 - Staining procedures and Appendices 3 and 6). PCR assay has been developed to detect *Cystoisospora belli* in stool samples[24].

Sarcocystis

Infections with *Sarcocystis* species are zoonotic in origin. *Sarcocystis* species differ from other coccidia in that they require two hosts to complete their life cycle. Humans become the intermediate hosts after eating undercooked meat of the primary host which contains sarcocysts. Most infections of this type are asymptomatic, but symptoms such as abdominal pain, nausea, bloating and diarrhoea can be associated with presence of sporocysts in the faeces. Muscular sarcocystosis results when humans become infected by eating undercooked meat or from handling infected cats. Symptoms then include muscle soreness or swelling.

Laboratory diagnostic techniques are the same as for *Cystoisospora* species, with mature sporocysts being present in the faeces from nine days post-infection. They cannot easily be distinguished from *Cystoisospora* species microscopically; therefore identification should be performed by a Reference Laboratory.

Toxoplasma

Toxoplasma gondii infection is asymptomatic in most immunocompetent patients; however symptoms of pyrexia, malaise, lymphadenopathy, encephalitis and myalgia may be experienced. Ocular disease also occurs. Infections may be primary or reactivations. Reactivation of latent infection occurs in patients who become severely immunocompromised. Foetal infection may result from an acute maternal infection during pregnancy. For more information on investigation of *Toxoplasma* in pregnancy, it is advised that the appropriate specialist Reference Laboratory should be contacted.

Laboratory diagnostic procedures include direct microscopic observation of *Toxoplasma gondii* tachyzoites in bronchoalveolar lavage fluid (BAL) stained with Giemsa stain and PCR. PCR has been found to provide better results especially in laboratories where there is less microbiological experience[25].

Alternative methods include serological tests, culture and histology. Serology is still the gold standard test used in organ transplant and HIV patients although these tests may give poor results in immunocompromised patients[25].

Cryptogregaria

Cryptosporidium

Cryptosporidium has now been formally reclassified from the Coccidia to a new subclass with gregarine parasites, Cryptogregaria, following an extensive review of molecular and biological data[26].

Cryptosporidium species can cause profuse watery diarrhoea in humans[27]. Children are particularly vulnerable due to lack of acquired immunity and poor personal hygiene. Infection shows seasonal variation with peak incidence in the spring and especially the autumn. Infection is a particular problem for patients who are severely immunocompromised[2]. The usual manifestation is severe, chronic diarrhoeal disease with signs of malabsorption, but other presentations include atypical gastrointestinal disease such as cholangitis, cholecystitis, pancreatitis and hepatitis. Respiratory tract disease has also been reported.

Primary laboratory diagnosis is based on stained microscopy (TP 39 - Staining procedures) or antigen detection by enzyme immunoassay followed by confirmation using microscopy stains or DNA detection by PCR[28,29]. Specialist tests include sensitive immunofluorescence microscopy and PCR-based tests for species/genotype identification which are available in the appropriate specialist Reference Laboratory. The sensitivity of modified Ziehl-Neelsen microscopy for detecting *Cryptosporidium* oocysts has been shown to be significantly less than for other tests[28-31].

Other organisms of uncertain taxonomic status

Blastocystis hominis

Previously described as a flagellate, a yeast, a coccidian, and an amoeba, this organism is part of a diverse group of organisms called stramenopiles[32]. The importance of *B. hominis* as a human pathogen is debatable[33]. Large numbers present in stools (more than five per high power field) may be associated with symptoms of nausea, abdominal pain, anorexia, flatus and diarrhoea[2].

Laboratory diagnosis by microscopy of primary wet smears (after formol-ether /ethyl acetate concentration), and smears stained with Giemsa and Field stain or differential staining kits may be used to maximise recovery of cyst-like forms[34] (see TP 39 – Staining procedures). Alternative methods include culture, PCR, ELISA and serological tests.

Culture techniques are, most likely, more sensitive than direct smears. Amplification of *Blastocystis*-specific DNA by polymerase chain reaction directly from stool has been reported and permits identification of the *Blastocystis* subtypes. However, serologic testing is not currently used for diagnosis of infection by this organism[35].

Microsporidia

There are over 150 genera and almost 1500 species of 'microsporidia'. The genera that are implicated in human diseases are *Anncaliia* (formerly *Brachiola*)*, Nosema, Enterocytozoon, Encephalitozoon, Microsporidium, Pleistophora*, *Trachipleistophora Tubulinosema* and *Vittaforma*[36]. There are at least 15 microsporidian species that have been identified as human pathogens: *Anncaliia algerae, Anncaliia connori, Anncaliia vesicularum, Encephalitozoon cuniculi, Encephalitozoon hellem*, *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi, Microsporidium ceylonensis*, *Microsporidium africanum*, *Nosema ocularum*, *Pleistophora* species, *Trachipleistophora hominis*, *Trachipleistophora anthropophthera*, *Vittaforma corneae*, and *Tubulinosema acridophagus*. These organisms are obligate intracellular organisms occurring in body fluids, tissues and the gastrointestinal tract. Originally classified as a parasite, these organisms are now known as fungi[37].

Microsporidiosis presents a particular problem in HIV-infected patients[2]. These and other immunocompromised patients are frequently infected with opportunistic parasites that do not usually produce symptoms in immunocompetent individuals[38]. Chronic diarrhoea is a major clinical feature in HIV infection and is a leading cause of morbidity and mortality[39]. Microsporidial keratoconjunctivitis has recently been recognised in patients with AIDS.

Histological stains, immunological techniques and electron microscopy may be used to identify these organisms in urine, sputum, bronchoalveolar lavages, bile, duodenal aspirates, faeces, tissues and scrapes from the cornea and conjunctiva. Microsporidia can be stained with modified trichrome stain (see TP 39 – Staining procedures Appendices 3 and 6).

Molecular identification of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (formerly *Septata intestinalis*), *Encephalitozoon hellem* and *Encephalitozoon cuniculi* on faeces can be done using species-specific PCR assays that are commercially available.

***Pneumocystis jirovecii* (formerly *Pneumocystis carinii*)[40]**

Pneumocystis jirovecii was thought to be a protozoan, but has been reclassified and is now considered to be a parasitic fungus based on nucleic acid and biochemical analysis[41]. It is an extracellular organism that causes interstitial plasma cell pneumonia found in humans, and generally causes disease in immunocompromised and immunosuppressed patients and in premature, sick and malnourished infants[42]. The symptoms of *Pneumocystis* pneumonia include dyspnea, non-productive cough, and fever.

P. jirovecii pneumonia is a common opportunistic infection of patients with AIDS (for more information, see B 57 – Investigation of bronchoalveolar lavage, sputum and associated specimens).

Laboratory diagnosis is made by staining specimens from induced sputum or bronchoalveolar lavage (BAL) for the microscopic identification of *P. jirovecii* trophozoites and cysts. This can be performed using Giemsa stain, a silver stain, or more specifically by direct or indirect immunofluorescent antigen detection (see TP 39 – Staining procedures). Molecular methods (PCR assays) have been shown to be extremely useful for the detection of *P. jirovecii* in clinical specimens[43,44].

Nematodes (roundworms)

The Nematodes belong to the phylum ‘Nematoda’. There are over 25,000 described species and of which many are parasitic. Laboratory diagnosis of nematode infestations relies mainly on the identification of eggs or larvae passed in the faeces. A wet preparation of faeces in saline following a formol-ether/ethyl acetate concentration permits the demonstration of ova microscopically (Appendix 5). The macroscopic presence of worms in the sample is also of diagnostic value.

Charcot-Leyden crystals in the stool can be seen with a wide range of parasitic bowel infections including amoebic dysentery. These crystals are hallmarks of eosinophil involvement in certain tissue reactions and are the result of eosinophil breakdown. Hexagonal bipyramidal crystals of Charcot-Leyden crystal protein can be seen by light microscopy or by fluorescence microscopy as bright yellow green fluorescing needles[45,46].

The occurrence of tissue and peripheral blood eosinophilia in travellers returning from long-term residence in, or a visit to, developing countries, or in immigrants from tropical areas, suggests the possibility of an infection with a helminth[47]. Some protozoal infections also cause eosinophilia. The greatest numbers of eosinophils in tissue and blood occur when the association of a parasite and host tissue is the closest, for example with migrating larvae or after their extended retention in tissue. Examples include trichinosis, visceral larval migrans, Ascaris pneumonia, strongyloidiasis, filariasis and acute schistosomiasis. Organisms, such as tapeworms, that remain in the bowel, and do not invade the intestinal mucosa, cause little or no eosinophilia.

Enterobius vermicularis

Also known as the thread or pin worm, it causes perianal and perineal pruritus, mainly in children[48]. Migrations of the parasite enable it to be found in the appendix, salpinges and in ulcerative lesions in the small or large bowel, but the causal relationship to clinical pathology is uncertain (Appendix 5).

Laboratory diagnosis is usually made by microscopy of a Sellotape preparation and/or perianal swab sample.

Trichuris trichiura

Also known as the whip worm, the infection can often be asymptomatic[48]. The loss of blood caused by adult worms embedding their heads in the intestinal mucosa is usually negligible, but heavy infections can cause a mild anaemia, bloody diarrhoea, dehydration, growth retardation and rectal prolapse (see Appendix 5).

Laboratory diagnosis is by identifying eggs in the faeces. In heavy infections, the stools are frequently mucoid and contain Charcot-Leyden crystals while in light infections, concentration methods are required for diagnosis.

Ascaris lumbricoides[47,48]

This nematode infection is usually asymptomatic. However the worms can cause serious pulmonary disease and obstruct the biliary and intestinal tracts. Larvae migrate through the lungs and may cause peripheral blood eosinophilia and symptoms associated with pulmonary infiltration. Third stage larvae may be seen in sputum. In children with heavy infections, the mass of worms can obstruct the lumen of the small

bowel. This causes abdominal distension, vomiting and cramps. It may also invade the biliary duct and cause epigastric pain, nausea and vomiting (Appendix 5).

Laboratory definitive diagnosis is usually made by microscopic examination of a faecal smear. Concentration techniques involving floatation or sedimentation of eggs may also be used. Diagnosis can also be made visually by the presence of the adult stage of *Ascaris lumbricoides* worm in freshly-passed faeces.

Hookworm

The two species of hookworm that cause human infection are *Ancylostoma duodenale* and *Necator americanus*[48]. Larvae penetrate the skin causing intense pruritus, erythema and a papular, vesicular rash. Larvae migrate through the lungs and may cause respiratory symptoms and eosinophilia in the sputum and peripheral blood. *A. duodenale* can cause infection by the oral route whereas *N. americanus* cannot. Symptoms include anaemia, chronic protein deficiency, abdominal pain, diarrhoea, weight loss and malabsorption. Hookworm eggs may be seen in faecal samples, but it is not possible to distinguish between species without hatching the eggs (see Appendices 5 and 8).

In older faecal samples infected with hookworm, first-stage larvae may be seen which must be differentiated from those of S*trongyloides* (Appendix 8).

Diagnosis is made by direct microscopic examination showing eggs in stool specimens. It should also be noted that the two species (that is, both *Ancylostoma duodenale* and *Necator americanus*) cannot be distinguished on the basis of their eggs but a formol-ether/ethyl acetate concentration method should be used in most cases.

Trichostrongylus species

They are also known as hairworms. These nematodes are distributed worldwide, but are rarely seen in Europeans. They may cause disease in humans and are associated with rural areas where herbivorous animals are raised. Infections in humans occur through ingestion of infective larvae from contaminated water or vegetables. Symptoms include abdominal pain, weight loss, diarrhoea, nausea, flatulence and generalised fatigue. Eosinophilia is seen frequently in symptomatic patients[49]. Diagnosis is made by direct microscopic examination showing eggs in stool specimens.

Strongyloides stercoralis

Strongyloides stercoralis invade the intestinal mucosa and deposit thin-walled eggs which hatch to rhabditiform larvae[47]. These larvae may be passed in the faeces or develop within the lumen of the bowel to infective larvae that can autoinfect the host. It may cause an overwhelming autoinfection in immunocompromised patients[48]. Pulmonary symptoms resemble those seen in hookworm infections. Other symptoms include burning or colicky abdominal pain, diarrhoea and passage of mucus, nausea, vomiting, weight loss, and malabsorption. Patients may also develop a generalised or localised urticarial rash beginning perianally and extending to the buttocks, abdomen and thighs.

First stage larvae (rhabditiform) are usually seen in faeces and eggs are only seen when diarrhoea is severe (see Appendix 8)[50]. Third stage larvae (filariform) can be seen in the sputum in cases of autoinfection. Direct smear or concentration methods of stool examination are useful for diagnosis of strongyloidiasis. However, serological

tests are a valuable aid in diagnosis. PCR has also been found very useful in the diagnosis of chronic *S. stercoralis* infections three to four weeks earlier than the currently used methods[51].

Unusual nematode infections

Laboratory diagnosis of the following diverse infections may be beyond the scope of most routine diagnostic laboratories. As many of these infections are rare and only occur in tropical countries, it is recommended that samples from patients be dealt with by a reference laboratory:

Trichinella species are ingested in raw or undercooked meat[47,52]. Most infections are asymptomatic, but large numbers of adult worms in the intestines can cause diarrhoea, abdominal discomfort and vomiting. Larvae burrow into skeletal muscles and cause fever, periorbital oedema and myositis with pain and swelling. Infection is confirmed by serology or a skin test for *Trichinella* specie. Muscle biopsy is usually unnecessary.

Visceral larval migrans (VLM) – This is also called Toxocariasis. Larvae migrate from the intestine to the liver, lung and trachea[47,53]. The most frequent agent is *Toxocara canis*. *Toxocara* species and other helminths (*Ascaris lumbricoides, Gnathostoma spinigerum*) may also be associated with the syndrome. VLM occurs mainly in children under six years of age. Most infections are asymptomatic, however patients may present with cough, fever, wheezing and hepatomegaly. Rarely, larvae may localise in the eye. Eosinophilia accompanied by leucocytosis is suggestive of VLM. Serological tests are available.

Ocular larval migrans (OLM) is the invasion of the eye by *T. canis* (and less frequently *T. cati*) larvae which become trapped in the eye and result in an eosinophilic inflammatory mass[53].

Cutaneous larva migrans, or creeping eruption, is commonly caused by *Ancylostoma braziliense*[53]. It presents as serpiginous, reddened, elevated, pruritic skin lesions. Other parasites such as *S. stercoralis* may also cause it.

Larvae of *Anisakis* species (associated with eating raw fish for example, sushi), *Phocanema* species and other genera, may penetrate the stomach and small intestine and cause abdominal symptoms which mimic appendicitis[53].

Laboratory diagnosis may be made by endoscopy, radiographic studies, or pathological examination of tissues. Serological tests are helpful in the diagnosis of Toxocariasis and Trichinellosis.

Angiostrongylus

Angiostrongylus cantonensis larvae can invade the brain and cause meningitis associated with eosinophilic pleocytosis in the CSF and peripheral eosinophilia[53]. The larvae may rarely be seen in the CSF. *Gnathostoma spinigerum* may cause a similar illness. Humans become infected through ingestion of larvae in raw or undercooked snails, or contaminated water and vegetables. Abdominal angiostrongyliasis is caused by *A. costaricensis*[53,54]. The larvae penetrate and develop in the lower small bowel and colon.

Laboratory diagnosis may be made by examining biopsy specimens.

Capillaria and *Paracapillaria*[42,53]

Capillariasis is a parasitic infection caused by new nematodes species namely, *Paracapillaria philippinensis* (previously known as *Capillaria philippinensis*) and *Capillaria hepatica* (also known as *Calodium hepaticum*)[55]*. P. philippinensis* may present as diarrhoea, vomiting, weight loss and malabsorption, while *C. hepatica* can cause acute/subacute hepatitis with peripheral eosinophilia and has similar symptoms as the former including ascites and hepatolithiasis. The main source of acquiring intestinal capillariasis is by eating of raw fresh water fish. This infection is seen in raw fish eating areas like the Philippines[56].

For the laboratory diagnosis of this infection, examination of faecal material by formol-ether/ ethyl acetate concentration or biopsy material is required. Alternative methods include immunodiagnosis which may be a supplementary diagnostic tool which helps to detect *P. philippinensis* infection as well as PCR which has been used successfully in the rapid diagnosis of *P. philippinensis,* thereby avoiding the delay in management and possible complications[56,57].

Dracunculus

Dracunculiasis (Guinea worm disease) is caused by the nematode parasite *Dracunculus medinensis* (known commonly as the Guinea worm). This infection is characterised by a chronic cutaneous ulcer from which the worm protrudes[52]. Some patients have a generalised reaction with urticaria, nausea, vomiting, diarrhoea and dyspnea. Painful ulcers develop which discharge fluid containing larvae. This may be examined microscopically to confirm the diagnosis.

Onchocerca volvulus

Onchocerciasis (known as river blindness) is caused by the filarial parasite *Onchocerca volvulus*. This parasite is transmitted by blackflies[52]. It causes an itchy dermatitis, subcutaneous nodules, keratitis and chorioretinitis. Laboratory diagnosis may be made by microscopic detection of microfilariae in blood and skin snips, indirect detection of microfilariae with the diethylcarbamazine patch test, detection of antibodies to onchocercal antigens, or detection of *O. volvulus* DNA in skin snips by PCR[58].

Adult worms can be found in nodule biopsy specimens (Appendix 13).

Mansonella streptocerca

This particular specie is transmitted by midges and blackflies[52]. Other species are generally found in blood. *Mansonella streptocerca* is usually diagnosed by finding microfilariae in blood and skin snips.

Dirofilaria immitis[53]

D. immitis causes lung nodules or subcutaneous abscess in humans. Migrating filariae die and cause local vasculitis which leads to pulmonary infarcts. Other species may cause subcutaneous masses. Laboratory diagnosis may be made by examining biopsies.

Trematodes (flukes)[59]

Generally, the laboratory diagnosis of trematodes is achieved by microscopic identification of eggs passed in the faeces; exceptions to this will be listed. Blood samples may be required especially when the parasite burden is low and stools and

urine samples may be negative. A wet preparation of the sample in saline followed by a formol-ether /ethyl acetate concentration permits the demonstration of ova microscopically.

Blood flukes

Schistosoma

There are five species of *Schistosoma*: *Schistosoma haematobium, Schistosoma intercalatum, Schistosoma japonicum, Schistosoma mansoni,* and *Schistosoma mekongi.* They may cause schistosomiasis[60]. The adult worms inhabit the portal and mesenteric blood vessels (except for *S. haematobium* which inhabits the vesical venous plexus). Major disease syndromes include a papular or urticarial rash, Katayama fever and chronic fibro-obstructive sequelae. These syndromes coincide with and are related to three different stages of development of the parasites in the host: cercariae; mature worms; and eggs. Penetrating cercariae cause a papular pruritic itch called swimmer's itch or schistosomal dermatitis. When worms have matured and begin depositing eggs, Katayama fever or acute schistosomiasis develops. Some eggs remain in the body of the host. These can lead to granuloma formation and tissue damage which may obstruct portal blood flow to the liver and pulmonary blood flow to the lungs, as well as urine flow through the ureters and bladder. Haematuria is the most common presentation of *S. haematobium* infection[47]. Chronic infection with *S. haematobium* can lead to bladder cancer[54].

Laboratory diagnosis is made by demonstrating eggs in the faeces (urine or semen for *S. haematobium*)[61]. Rectal snips or biopsies may also be examined. Serological tests can be of value when eggs cannot be found in clinical samples. PCR has been found to be very valuable for diagnosis in the early phase of schistosomiasis[62].

Liver flukes

Opisthorchis sinensis (formerly known as *Clonorchis sinensis*)

Also known as the Chinese liver fluke. Clonorchiasis is an infection caused by *Opisthorchis sinensis*. These may cause localised obstruction of the bile ducts and thickening of the walls in heavy infections, as well as cholangitis and cholangiohepatitis[60].

The standard diagnostic method is microscopic examination of faeces / duodenal aspirate thereby identifying eggs. Alternative methods include ELISA, which has become the most important method. Detecting DNA from eggs in faeces can be done using PCR and LAMP assays, which are highly sensitive and specific. Imaging diagnosis is also to be very useful and is now widely used[63].

Opisthorchis viverrini and Opisthorchis felineus

Also known as Southeast Asian liver fluke and cat liver fluke respectively. These liver fluke parasites generally cause the infection, Opisthorchiasis and this is acquired by humans ingesting raw or undercooked infected fish which contain metacercariae of *O. viverrini.*

Symptoms caused include abdominal pain, diarrhoea or constipation. Chronic symptoms include obstruction of the biliary tract, inflammation and fibrosis of the biliary tract, liver abscesses, pancreatitis, and suppurative cholangitis. In rare cases, they cause liver cholangiocarcinoma[59].

Diagnosis is as shown above in *Opisthorchis sinensis*.

Fasciola hepatica

Infestation has two distinct clinical phases corresponding to a hepatic migratory phase of the life cycle and to the presence of worms in their final habitat, the bile duct[60]. The early phase may present with fever and pain in the upper quadrant. Later, biliary obstruction may occur.

Ova may be demonstrated in the faeces or the bile. Serological tests are also available.

Intestinal flukes

These include *Fasciolopsis buski, Heterophyes heterophyes, Nanophyetus salmincola, Metagonimus yokogawai* and *Echinostoma* species[53,60]. Most *F. buski* infections are asymptomatic. Heavy infections can cause diarrhoea, abdominal pain and malabsorption. *H. heterophyes* causes abdominal pain and diarrhoea. *Echinostoma* species infection is rare but has been documented. *N. salmincola* causes diarrhoea, abdominal pain, bloating and eosinophilia.

Ova and parasites may be demonstrated in the faeces.

Lung flukes

Paragonimus species

There are almost 50 species and subspecies of *Paragonimus*, of which over 13 of these are known to infect humans causing the condition, “paragonimiasis”. The most common is *Paragonimus westermani*, also known as the ‘oriental lung fluke’. Humans get infected when they eat uncooked or undercooked seafood such as freshwater crabs or crayfish that contain the parasites. They encapsulate within the lung parenchyma, usually close to the bronchioles[60]. Eggs are deposited which pass into the bronchioles and are coughed up. These may then be detected in the sputum or, if they are swallowed, in the faeces. Patients develop eosinophilia and experience chest complaints; they may cough up brownish sputum and have intermittent haemoptyses. This leads on to chronic bronchitis or bronchiectasis with profuse expectoration and pleuritic chest pain.

Laboratory diagnosis may be made microscopically by demonstrating the presence of eggs (ova) in the sputum or faeces. Other alternative tests include EIA and serology tests, which has been useful for diagnosis in early infections[64].

Cestodes (tapeworms)

Generally, the laboratory diagnosis of cestodes is achieved by microscopic identification of eggs passed in the faeces. Exceptions to this will be listed. A wet preparation of sample in saline followed by a formol-ether concentration permits the demonstration of ova microscopically. The macroscopic presence of proglottids in the sample is also of diagnostic value (Appendix 5 and 7).

In humans, cestode infestations occur in one of two forms: mature tapeworms within the gastrointestinal tract, or as one or more larval forms (called hydatidosis, cysticercosis, coenurosis and sparganosis) embedded in the liver, lung, muscle, brain, eye or other tissues.

Diphyllobothrium latum

Also known as the fish tapeworm, it is associated with cold, clear lakes found in Scandinavia, northern Europe, northern Japan, Canada, Alaska and North America. It causes a parasitic infection called 'diphyllobothriasis' in humans which is acquired by eating raw fish infected with the parasite. Infestations are usually asymptomatic, however when the parasite reaches a large size it may cause mechanical obstruction of the bowel resulting in diarrhoea and abdominal pain. Prolonged or heavy infections cause vitamin B12 deficiency. In some cases it can lead to neurological symptoms[65].

Hymenolepsis nana and related cestodes

Hymenolepsis nana is the smallest of the tapeworms that infect humans. It is also known as the 'dwarf' tapeworm due to its particularly small size. It is the most common tapeworm infection of humans worldwide[65]. This infection may cause mild abdominal discomfort, irritability, anorexia and diarrhoea. Although humans may acquire infection by accidental ingestion of infected beetles (often found in dry cereals), direct infection is more common and usually occurs in familial and institutional settings where hygiene is poor. *Hymenolepsis diminuta*, primarily a parasite of rats, is an occasional human parasite via the ingestion of beetles in cereals. *Dipylidium caninum* is commonly found in dogs and cats. Children in particular may become infected through close contact with the animals and their fleas.

Diagnosis can be achieved by recovery and identification of the characteristic ova in a formol-ether concentrate of faeces. Adult worms and proglottids are rarely seen in stool samples.

Taenia saginata

Also known as the beef tapeworm. It is more common in developing countries where hygiene is very poor. Humans become infected when raw or undercooked meat is ingested. They cause abdominal discomfort and patients experience proglottid migration from the anus[65].

Taenia solium

Also known as the pork tapeworm. The adult worms cause minimal symptoms, but the larval cysts cause local inflammation[65]. Migration of these into the central nervous system causes seizures, hydrocephalus and arachnoiditis.

Cysticercosis (this is tissue infection with cysticerci of *T. solium*) can develop in humans by autoinfection from the adult worm. Involvement of the central nervous system is called neurocysticercosis[65].

Diagnosis can be achieved by stool microscopy, serology, immunodiagnostic methods or biopsy[66].

Taenia multiceps, *Taenia serialis* and *Taenia brauni*

Taenia multiceps and *Taenia serialis* are more commonly seen in Europe and USA while *Taenia brauni* is common in Africa. These species cause a cyst infection called coenurosis, and usually develop into tapeworms in dogs[65]. Symptomatic disease in humans involves the eye, central nervous system, sub-cutaneous and muscular tissues. Laboratory diagnosis is by microscopic examination of appropriate material for the presence of protoscolices (similar to those of *T. solium*).

Spirometra mansonoides

The tissue infection, sparganosis is usually caused by *Spirometra mansonoides*. It is a tissue infection with plerocercoid larvae of several different cestode species, and symptoms include local inflammation of the skin at the site of invasion[65]. Tissue injury may be severe, particularly in the eye, because some forms of the parasite spread to other areas of the body.

Laboratory diagnosis is by histological techniques only.

Echinococcus granulosus, Echinococcus vogeli and Echinococcus multilocularis

These tapeworms cause the parasitic disease called Echinococcosis which has two forms: cyst echinococcosis caused by *Echinococcus granulosus* or *Echinococcus vogeli*, and alveolar echinococcosis caused by *Echinococcus multilocularis*[65]. Eggs hatch to form oncospheres that penetrate the gut mucosa and enter the circulation. These encyst in the host viscera and develop to form mature larval cysts. Symptoms occur due to the mechanical effects of enlarging cysts in confined spaces.

Laboratory diagnosis can be made by:

1. Imaging and serodiagnosis are the mainstay of diagnosis. Serological tests include Enzyme linked immunosorbent assay (ELISA), an indirect haemagglutination test and a complement fixation test

2. In the case of cyst echinococcosis, microscopic examination of the cyst fluid to look for the characteristic protoscolices which can be either invaginated or evaginated. The cyst fluid may also reveal free hooklets

Note: Diagnostic aspiration should only be undertaken in a specialist unit with experience in managing such parasites.

3. Histological examination of the cyst wall after surgical removal

Occasionally pulmonary cysts containing *E. granulosus* may rupture and intact protoscoleces and hooks can be coughed up in the sputum, and seen in microscopic preparations. Serological tests are also available.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers[67,68]

UK SMIs use the term, "CE marked leak proof container," to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes."

Rapid diagnostic tests

A variety of rapid identification methods of varying sensitivities and specificities are available. These techniques may have potential advantages/disadvantages and should therefore be evaluated and validated prior to use. Molecular methods (for example, multiplex PCR) and enzyme immunoassays (EIA) may perform better than conventional methods, and should therefore be considered for use where available, following validation to ensure appropriate clinical interpretation[30,69].

Commercial test kits

The quality of reagents in commercially available test kits may be variable or deteriorate under storage conditions; for that reason in-house as well as external controls are necessary to determine whether the kit is fit for purpose. Laboratories should use test kits according to manufacturers' instructions.

Concentration methods

Faecal specimens should be submitted fresh, without formalin, as concentration is not required routinely prior to staining. Where concentration is deemed necessary, modified methods should be used to minimise oocyst losses and prevent interference with the adhesion of oocysts to slides and with staining.

Toxoplasma testing

Several commercial kits for *Toxoplasma* serologic testing are available. However, the sensitivity and specificity of these kits may vary widely from one commercial brand to another. This is of concern because serology results can influence decisions on continuation or termination of pregnancies.

Enzyme immunoassays (EIA)

Specimens (faeces) to be tested using EIA or other rapid assays should not be concentrated prior to testing because antigens (such as those targeted in diagnosis of *Giardia intestinalis* and *Cryptosporidium* species) are lost during the procedure such as in diagnosis of *Giardia intestinalis* and *Cryptosporidium* species. Most EIAs require the use of fresh or frozen stool specimens[28]. However, there are now some commercially available test kits that use preserved faecal specimens for detection of antigens, and users should check the manufacturers' instructions.

Preservation of specimens

Preservation in 10% formalin is necessary when faeces cannot be examined within the prescribed time interval. These specimens can be stored for several months. However it should be noted that it has its drawbacks which include its interference with PCR especially after extended fixation time, its inadequate preservation of morphology of the protozoan trophozoites as well as its non-suitability for some smears stained with trichrome stain[70].

Problems with identification

There are many microscopic artefacts that can be found in faeces which may be confused with trophozoites, (oo)cysts or eggs. See Appendix 4 for more information.

Problems with microscopy

The diagnosis of *E. histolytica* infection has always relied on microscopic examination of fresh or fixed stool specimens however, microscopy has its limitations which include its suboptimal sensitivity which is about 60%, and secondly, its inability to distinguish potentially pathogenic *E. histolytica* from morphologically identical but non-pathogenic *E. dispar*, *E. moshkovskii*, and other quadrinucleate cysts of *Entamoeba*[3,4].

Other drawbacks to microscopy generally include its tediousness when large numbers of specimens need to be examined and the lack of microscopic expertise among laboratory staff.

1 Safety considerations[67,68,71-85]

1.1 Specimen collection, transport and storage[67,68,71-74]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags, with the exception of Sellotape slide/perianal swab for *E. vermicularis* ova which should be transported in a sealed plastic bag[1].

In the case of CSF, any inoculated plates should also be transported in a robust, CE marked leak proof container.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[67,68,71-85]

All sample types

Containment Level 2 unless otherwise specified (see below).

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[77].

Disposable gloves should be worn for all parasitology investigations.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

Faeces

Containment Level 3 is not required for investigation of *Echinococcus* species and *Taenia solium* but a Class 1 microbiological safety cabinet is essential.

Unpreserved tapeworm proglottids submitted to the laboratory for identification are hazardous due to the possibility of accidental infection, and the possibility of cysticercosis[86].

Care should be taken even with faecal specimens that have been fixed in preservatives as they could potentially be infectious. Fixation in formalin can take days to weeks to kill some parasite cysts or oocysts, for example, eggs of *Ascaris lumbricoides* may continue to develop and are infectious even when preserved in formalin.

Specimens for microscopy only should be prepared in 10% (v/v) formalin in water (this would not be suitable for the examination of trophozoites).

For formol-ether concentrations, ethyl acetate should be used in place of diethyl ether for safety reasons[42]. Procedures should be carried out in a well-ventilated area with no naked flames.

CSF

Containment Level 3 and a safety cabinet are required for the investigation for *Naegleria fowleri.*

Tissues, biopsies, hydatid cyst and pus from abscesses

Process specimens from the lung and pleural cavity, and hydatid cysts, in a Class 1 exhaust protective cabinet in a Containment Level 3 room.

Sputum/bronchoalveolar lavage

All specimens must be processed in a Class 1 exhaust protective cabinet in a Containment Level 3 room.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Faeces, urine, sellotape slide/perianal swab, CSF, bile, pus from abscesses, duodenal/jejunal aspirates, tissues, hydatid cyst, sputum/bronchoalveolar lavage, biopsies from colonoscopy or surgery

2.2 Optimal time and method of collection[87]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[87].

Faeces

Faeces should be presumably collected before antimicrobial or anti-diarrhoeal therapy where possible and between 10pm and midnight, or early in the morning, before defecation or bathing.

Sellotape slide/perianal swab should be collected for *E. vermicularis* ova[1].

Fresh faeces specimens are essential for the examination of trophozoites.

Faeces may be passed directly to a sterile wide-mouthed CE marked leak proof container or may be passed to a clean, dry bedpan or similar container and transferred to a CE marked leak proof container.

Fresh, unpreserved specimens should be transported immediately. Cysts will not form once the specimen has been passed.

Protozoan trophozoites will not survive if the specimen dries out. Use of 10% formalin will kill trophozoites and renders them immotile. Liquid stool should therefore be examined ideally within 30 minutes from the time of collection without the addition of formalin (usually with a drop of saline) if trophozoites are sought. If delays cannot be avoided, the specimen should be preserved to avoid disintegration of the trophozoites.

Soft stools (which may contain both trophozoites and cysts) should preferably be examined within 1hr of passage[88].

Formed specimens (less likely to contain trophozoites) can be kept for up to one day, with overnight refrigeration if needed, prior to examination[89].

Microscopy for *E. vermicularis* ova[1]

Sellotape slide

Apply clear Sellotape to the perianal region, pressing the adhesive side of the tape firmly against the left and right perianal folds several times; the tape can be wrapped around a tongue depressor to aid specimen collection. Smooth the tape back on the slide, adhesive side down.

Perianal swab

Perianal specimens are best obtained in the morning before bathing or defecation. Three specimens should be taken on consecutive days before pinworm infection is ruled out.

Cotton-wool swab in dry container should be used for collection.

Spread buttocks apart, and rub the moistened cotton wool swab over the area around the anus, but do not insert into the anus. Place cotton wool swab back in its container (no transport medium required). Occasionally, an adult worm may be collected from a patient and sent in saline or water for identification.

Urine (for *S. haematobium*)

In urinary schistosomiasis, very few ova are present in the urine. The number of ova in the urine varies throughout the day, being highest in urine obtained between 10am and 2pm[90]. In patients with haematuria, eggs may be found trapped in the blood and mucus in the terminal portion of the urine specimen. It is therefore preferable to obtain total urine collected over the time period between 10am and 2pm[91]. Alternatively, a 24hr collection of terminal samples of urine may be helpful. Sterile containers without boric acid must be used[90].

If the urine cannot be examined within an hour of collection, it is advisable to add 1mL of undiluted formalin to preserve any eggs that may be present[90].

CSF

Specimens will be obtained by specialist collection according to local protocols.

Tissues, biopsies, hydatid cyst and pus from abscesses, bile, duodenal/jejunal aspirates

Specimens will be obtained by specialist collection according to local protocols.

Sputum/bronchoalveolar lavage

Sputum from the lower respiratory tract expectorated by deep coughing is required. When the cough is dry, physiotherapy, postural drainage or inhalation of an aerosol before expectoration may be helpful.

2.3 Adequate quantity and appropriate number of specimens[87]

Faeces

Ideally three stool specimens collected over no more than a 10-day period. It is usually recommended that specimens are collected every other day. Unless the patient has severe diarrhoea or dysentery, no more than one specimen should be examined within a single 24 hour period, as shedding of cysts and ova tends to be intermittent.

If *E. histolytica* is suspected and the first three specimens are negative, consideration should be made for referral where available for molecular tests.

There are no prescribed limits for the size of sample required, as some laboratory procedures will require larger quantities than others.

Sellotape slide/perianal swab for *E. vermicularis* ova[1]

It is recommended that samples should be taken for at least four to six consecutive days. If the results of all these are negative the patient can be considered free from infection. In practice, more than one specimen is rarely received.

Urine (for *S. haematobium*)

Ideally, a minimum volume of 10mL is required.

CSF

Ideally, a minimum volume of 1mL is required.

Tissues, biopsies, hydatid cyst and pus from abscesses

Pus

Ideally, the entire volume of pus or a minimum of 1mL is required.

Tissues/biopsies

Ideally, the specimen should be large enough to carry out all investigations required.

Bile, duodenal/jejunal aspirates

Ideally, a minimum volume of 1mL is required.

Sputum/bronchoalveolar lavage

Ideally, a minimum volume of 2mL is required.

3 Specimen transport, storage and retention[67,68]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[87].

Samples should be retained in accordance with The Royal College of Pathologists guidelines ‘The retention and storage of pathological records and specimens’[92].

Faeces

If prompt examination of stools cannot be carried out, the use of 10% formalin-water preservative is necessary to prevent deterioration of protozoan morphology, the hatching of first-stage hookworm larvae, and overgrowth of yeasts[93].

Sellotape slide/perianal swab for *E. vermicularis* ova[1]

Refrigeration or store at room temperature (20 - 25°C) for up to 48hr.

Urine (for *S. haematobium*), bile, duodenal/jejunal aspirates and sputum/bronchoalveolar lavage

If processing is delayed, refrigeration is preferable to storage at room temperature (20-25°C).

Tissues, biopsies, hydatid cyst and pus from abscesses

If specimen of tissue/biopsy is small, place it in sterile water to prevent desiccation.

If processing is delayed, refrigeration is preferable to storage at room temperature (20-25°C).

4 Specimen processing/procedure[67,68]

4.1 Test selection

Faeces

Select a representative portion of specimen for appropriate procedures such as culture for bacterial pathogens (B 30 -Investigation of faecal specimens for enteric pathogens), testing for Clostridium difficile toxins (B 10 – Laboratory investigation of *Clostridium difficile* infection) and virological examination, depending on clinical details.

Faecal concentrations are carried out on all specimens where examination of parasites is specifically requested, where there are definite clinical indications and when advised by senior laboratory staff.

All faecal samples from symptomatic individuals should be tested for *Cryptosporidium* oocysts[94].

Stain for microsporidia in symptomatic, HIV positive and immunocompromised patients.

Incubation period and life cycle of individual parasitic infections should be determined in test selection.

For all other specimens

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2. Follow manufacturers' instructions if commercial kits are used.

4.3.1 Pre-treatment

N/A

4.3.2 Specimen processing

Faeces

Sample parts of faeces samples that contain blood, pus, or mucus for direct examination as wet preparations or for staining.

If sampling formed faeces, collect and examine material from various parts of the faecal sample for concentration, wet preparations and for staining.

Faeces for microscopical examination of protozoa

Standard

If specimen is fresh, examine for motile trophozoites as follows:

1. Place one drop of 0.85% saline on the left-hand side of a clean microscope slide, and one drop of double-strength Lugol's iodine on the right-hand side (the distance between the drops should be sufficient to enable coverslips to be placed over each drop).
2. Using a different swab stick for each preparation, take a small amount of unfixed faeces and thoroughly emulsify in the saline and in the Lugol's iodine.
3. Place cover slips over each preparation on the slide. Examine both entire areas with a low power objective. Use a medium power objective to identify any suspicious morphological features.
4. If required, prepare smears on clean microscope slides for auramine-phenol and/or Giemsa staining (see TP 39 - Staining procedures).
5. Also, concentrate the specimen with the formol-ether* concentration technique described below.

Faeces for examination of *Cryptosporidium* species[88,95]

1. Prepare a medium to thick smear of faeces on a clean microscope slide and air dry.

   Note: If specimen is dry or solid, 10% formalin may be added.
2. Fix in methanol for three minutes.
3. Smears can be stained by either auramine phenol or modified cold Ziehl-Neelsen (see TP 39 - Staining procedures).

Modified formol-ether* concentration[96,97]

***Ethyl acetate (not diethyl ether) must be used in a well-ventilated area with no naked flames**

The following method is the recommended technique for faecal concentration. There are many commercial kits for the concentration of faeces available which are based on the Ridley Allen method described below, which is the method of choice used by most clinical laboratories. Commercial concentration kits are often used.

1. Take a sample of faeces about the size of a large pea (approximately 1g) with a swab stick and emulsify it in 7mL of 10% formalin (one volume of 40% formaldehyde diluted with nine volumes of distilled water) in a clean universal container.
2. Sieve by pouring the whole contents of the universal through a sieve (a nylon tea-strainer or a square of wire gauze is suitable) and collect in a suitable container. Sieves are washed in copious amounts of clean water and re-used. Sieving the faeces and formalin mixture prior to centrifugation helps eliminate large pieces of faecal matter from the suspension.
3. Transfer the filtrate into a stoppered glass or polypropylene (ether resistant) container appropriate for centrifugation.
4. Add 3mL of ethyl acetate and a small drop of 0.1% Triton X 100 (helps emulsify the faecal specimen) and vortex for 15secs, or shake vigorously for 60secs.

5. Centrifuge the specimen at 1200 x g for 3min.
6. Loosen the fatty layer with a swab stick by passing it around the inside circumference of the tube, removing all residues of the fat from the tube.
7. Tip away the contents of the tube, allowing the last few drops to return to the bottom of the tube to cover the remaining deposit.
8. Resuspend the deposit in the remaining fluid. Place a drop of this on a clean microscope slide and place a coverslip over it.
9. Double strength iodine may be added to a separate preparation to enhance and facilitate comparison of morphological details.
10. Search the entire area using a low power objective; use a medium power objective to examine morphological features.

Commercially available concentrator kits containing sieves of varying pore sizes are available; the size of the pore affects the yield of parasite stages and the amount of debris present[98-101]. A larger pore size may result in a higher yield of parasite stages, however the increase in debris leads to a denser deposit, making it more difficult to examine the slide; ova and cysts may therefore be obscured. If the pore size is too small, despite having a cleaner slide which is easy to examine, the yield of parasite stages will be reduced. Commercially available faecal concentrator kits should be validated prior to use, and manufacturers' instructions should be followed.

To maximise the recovery of parasites it is important to sieve the faecal formalin mixture, use a solvent, that is, ethyl acetate with triton X and centrifuge for the correct time and at the correct centrifugal force[99]. Recovery of parasite stages may be greatly diminished if a solvent (for example ethyl acetate) as an extractor of fat and debris is not used[101]. A recent study confirms and recommends that 1200 x g for 3min is optimal for parasite recovery[101,102].

Sellotape slide

1. Before examining the slide, it may be advantageous to lift the tape and place one drop of immersion oil or glycerol/alcohol under the middle of the tape and replace in position. This will improve the transparency of the tape.
2. Examine the slide using a low power objective.

Perianal swab

1. Add enough saline to cover the swab in its container and replace the cap.
2. Shake vigorously.
3. Withdraw the swab from the saline, rolling it against the side of the container to squeeze out the saline. Discard the swab.
4. Concentrate the resultant fluid by centrifugation at 800 x g for 2min.
5. Remove the supernatant with a disposable pipette, without disturbing the sediment.
6. Agitate the tube to resuspend the residue.
7. Using a disposable pipette, place a drop of sediment on a microscope slide, apply a coverslip and use a low power objective to examine the entire area.

Urine (for *S. haematobium*) [90]

A complete urine sample collected between 10am – 2pm should be submitted. Alternatively, submit terminal stream urines collected over a whole 24 hour period.

For large volumes of urine (>25mL)

Sedimentation method

1. Allow the specimen to sediment for 1hr.
2. Decant and discard the supernatant, then transfer the sediment along with some residual urine (approximately 1mL) to conical-bottom containers for centrifugation.
3. Centrifuge at 500 x g for 2min.
4. Decant and discard the supernatant then mix the sediment using a pipette.
5. Place 1- 2 drops of the whole deposit on several clean microscope slides and apply coverslips.
6. Examine the entire area of each slide preparation with a low power objective.

Other urine specimens

If specimen is already in a conical-bottom container, proceed as from number 3 above, if not, transfer the entire specimen to conical-bottom containers and proceed as from number 3 above.

Filtration method (recommended for non-cellular, crystal-free urine specimens)

1. Draw ≥10mL urine into a syringe, and then connect to a Swinnex filter (pore size 12µm).
2. Gently ease the urine through the filter.
3. Draw in 20mL of air and ease this through the filter.
4. Remove the top of the filter and place the membrane on a microscope slide.
5. Add a drop of saline, apply a coverslip and view microscopically with a low power objective.

CSF[103]

1. Perform direct microscopy of the CSF as soon as received (use a low or medium power objective).
2. Concentrate the CSF by centrifuging at 100 x g for 10min.
3. With a sterile pipette transfer the supernatant, leaving approximately 0.5mL, to another sterile container for additional testing if required (protein content, virology, etc.).
4. Resuspend the centrifuged deposit in the remaining fluid and place two drops in the centre of a bacteria-coated agar plate.
5. After the fluid has been absorbed, incubate and examine the plate as described for corneal scrapings (refer to B 2 - Investigation of bacterial eye infections).

Note: 35°C–37°C incubation will give better results for *Naegleria* species.

1. Also, place one drop of the centrifuged deposit on a clean microscope slide and place a cover slip over it. Screen the entire area with a low power objective; use a medium objective to identify any morphological features.

Hydatid cyst and pus from abscesses

For pus and hydatid cyst contents (including hydatid sand), prepare wet preparations and air-dried smears for staining (if required) (see TP 39 - Staining procedures).

Tissues and biopsies[103]

1. In addition to standard histological preparations make impression smears, teased and squash preparations.
2. Place specimen in a sterile Petri dish to examine it macroscopically and to select a sample for microscopic examination.
3. Select an area that appears to look different from normal. For example, select grey consolidated or granulomatous portion of a lung, liver or the ulcerated area of intestinal tissue.

Impression smears

1. If the sample is large enough, cut the tissue and use the cut surfaces to touch the slide.
2. Press the tissue against a clean microscope slide, lift and press again.
3. Turn the sample over and press the area of the cut surface against the slide to make two more impressions. This leaves three impressions in a row on one microscope slide.
4. If several tissue samples were supplied, make a row of impressions with each of the samples.
5. Air dry and fix in methanol for 1min before staining by Giemsa (see TP 39 - Staining procedures).

Squash preparations

Squash preparations - for tissue parasites such as *Trichinella*:

1. Cut selected tissue portions into very fine fragments in a Petri dish, placing a fragment on a clean microscope slide.
2. Add one drop of sterile saline or sterile distilled water.
3. Cover with a second clean microscope slide and press the slides firmly together.
4. Examine microscopically with a low power objective.

Note: Care should be taken when squash preparations are performed as they can release lots of eggs increasing the risk to the operator.

Bile, duodenal/jejunal aspirates

Standard

1. Centrifuge specimen at 800 x g for 2min.
2. Decant supernatant to disinfectant.
3. Resuspend the centrifuged deposit in the remaining drops of supernatant.

4. Use the resuspended pellet to prepare smears for staining.
5. Stain using Ziehl-Neelsen and auramine-phenol for *Cryptosporidium*, Giemsa for *Cyclospora cayetanesis* and *C. belli* (see TP 39 - Staining procedures), and iodine or plain wet preparation for *S. stercoralis* and *G. duodenalis.*

Supplementary

For examination of microsporidia:

1. Centrifuge specimen at 800 x g for 2min.
2. Decant supernatant to disinfectant.
3. Resuspend the centrifuged deposit in the remaining drops of supernatant.
4. Use the resuspended pellet to prepare smears for staining.
5. Stain with the modified trichrome stain (see TP 39 - Staining procedures).

Sputum[103]

1. Select any blood-tinged viscous areas for sampling.
2. Place 1mL of sputum in a centrifuge tube.
3. Add 1mL dithiothreitol and agitate gently for approximately 10sec. Allow to stand at room temperature for 15min.
4. Centrifuge at 1500 x g for 2min.
5. Decant the supernatant to a discard jar.
6. Resuspend the deposit in the few remaining drops of supernatant.
7. Place one drop of this on a clean microscope slide and apply a cover slip. Examine the entire area with a low power objective.

Induced sputum / BAL (for *P. jirovecii*)

Several methods exist for the staining and identification of *P. jirovecii*. Histological stains may be used, however immunofluorescent methods with monoclonal antibodies are used in many microbiology laboratories. Manufacturers' recommendations should be followed when using commercial kits and reagents. For more information, see B 57 – Investigation of bronchoalveolar lavage, sputum and associated specimens.

4.4 Microscopy

See Section 4.3.2 for all specimens.

4.5 Culture and investigation

See Section 4.3.2 for all specimens.

4.6 Identification

Minimum level

Identify parasites to species level and their stages where possible.

4.7 Antimicrobial susceptibility testing

N/A

4.8 Referral for outbreak investigations

For information regarding outbreak investigation referral, contact specific reference laboratory.

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Report on any parasites seen.

Faeces

Include comment on the presence of all stages of parasites seen, whether they are pathogenic or non-pathogenic.

Microscopy reporting time

Written report 16–72hr stating, if appropriate, that a further report will be issued.

Urgent microscopy: telephone when available.

5.2 Culture

CSF

Report presence or absence of *Acanthamoeba* species and/or *Naegleria fowleri.*

Culture reporting time

CSF

Written report on day four stating if appropriate, that a further report will be issued.

Clinically urgent requests: telephone when available.

5.3 Antimicrobial susceptibility testing

N/A

6 Notification to PHE[104,105], or equivalent in the devolved administrations[106-109]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[106,107], Wales[108] and Northern Ireland[109].

Appendix 1: Specimen types and possible parasites detectable

Specimen types	Possible parasites present
Bile	*Fasciola hepatica, Opisthorchis sinensis, Cryptosporidium* species
CSF	*Acanthamoeba* species, *Angiostrongylus cantonensis*, *Balamuthia mandrillaris,* Microsporidia - *Encephalitozoon cuniculi, Naegleria fowleri,* any nematodes producing VLM (Visceral Larva Migrans), cestodes, *Taenia solium, Echinococcus* species
Duodenal and jejunal aspirates	*Cryptosporidium* species*, Cyclospora cayetanesis, Giardia duodenalis* Microsporidia - *Enterocytozoon bieneusi*, *Strongyloides* species
Faeces	*Ancylostoma duodenale, Ascaris lumbricoides,* adult *Acaris* sp. worms and ova, *Balantidium coli*, *Blastocystis hominis, Paracapillaria philippinensis, Chilomastix mesnili, Cryptosporidium* species, *Cyclospora cayetanesis, Dientamoeba fragilis, Diphyllobothrium latum, Echinostoma* species, *Endolimax nana*, *Entamoeba histolytica*, other *Entamoeba* species, *Enteromonas hominis, Fasciola hepatica, Fasciolopsis buski, Giardia duodenalis, Heterophyes heterophyes, Hymenolepis nana*, *Iodamoeba butschlii, Cystoisospora belli*, Microsporidia [*Enterocytozoon bieneusi and Encephalitozoon (Septata) intestinalis*], *Metagonimus yokogawai*, *Nanophyetes salmincola, Necator americanus, Opisthorchis sinensis*, *Paragonimus* species*, Retortomonas intestinalis, Sarcocystis* species, *Schistosoma* species *Strongyloides stercoralis, Taenia saginata* worms and ova*, Taenia solium*, *Trichuris trichiura, Enterobius* species adult worms and ova
Liver and spleen aspirates Hydatid cyst Pus	*Entamoeba histolytica, Leishmania* species, *Echinococcus granulosus, Fasciola hepatica*
Sellotape slide	*Enterobius vermicularis*
Sputum / BAL	*Ascaris lumbricoides*, *Cryptosporidium s*pecies, Microsporidia, *Paragonimus westermani, Strongyloides stercoralis, Pneumocystis jirovecii*
Swabs	*Trichomonas vaginalis* – genital swab Microsporidia – eye swab
Tissues and biopsies	*Acanthamoeba* species - brain biopsy, skin nodules and ulcers; *Angiostrongylus costaricensis*, *Anisakis* species, *Cryptosporidium* species – small bowel and liver biopsy; Filarial worms, *Giardia duodenalis* - duodenal biopsy; *Leishmania* species - lymph node biopsy, cutaneous ulcers; *Anncaliia* species*, Nosema* species*, Vittaforma corneae, Microsporidium africanum and Microsporidium ceylonensis* - Cornea ulcer Microsporidia [*Pleistophora, Nosema*, *Trachipleistophora* and *Phocanema* species], *Schistosoma* species, *Taenia solium*, *Trichinella* and other tissue nematodes - muscle biopsy
Urine	*Schistosoma haematobium,* Microsporidia

Serology testing	*Entamoeba histolytica, Acanthamoeba* species, Cysticercosis (*Taenia solium*), *Echinococcus granulosus, Fasciola hepatica*, Filaria, *Cryptosporidium* species, *Leishmania* species, *Schistosoma* species, *Strongyloides stercoralis*, *Toxocara* species, *Toxoplasma gondii*, *Trichinella* species, any nematodes producing VLM (Visceral Larva Migrans),

Appendix 2: Geographic distribution of parasitic infections

Infection / Infective organism	Geographic distribution
Amoebiasis/ *Entamoeba histolytica*	Worldwide
Free-living amoebae - *Acanthamoeba*, *Naegleria* sp.	Worldwide
Flagellates:	
Giardiasis, Trichomoniasis	Worldwide
Coccidia:	
Cryptosporidiosis/*Cryptosporidium* sp.	Worldwide
Cyclosporiasis/*Cyclospora* sp.,	Worldwide particularly high incidence areas such as Mexico, Honduras,
Microsporidia	Worldwide
Cystosporiasis/*Cystoisospora* sp.,	Worldwide
Sarcocystosis/*Sarcocystis* sp., Toxoplasmosis/ *Toxoplasma gondii*, *Pneumocystis jirovecii*	Worldwide
Nematode infections (GI-tract) – *Enterobius, Trichuris, Ascaris*	Worldwide
Hookworms:	
Ancylostoma duodenale	Europe, S America, India, China, SE Asia, Indonesia, Australia, some Pacific isles
Strongyloides sp.	Tropics and subtropics
	Worldwide
Trichostrongylus species	
Necator americanus	N and S America, sub-Saharan Africa, India, China, SE Asia, Indonesia, Australia some Pacific isles
	Worldwide
Unusual nematodes:	Asia
Trichinellosis, Toxocariasis	
Gnathostomiasis (fever and pulmonary infiltrates)	Africa, America, Asia, Australasia
Gnathostomiasis, *Angiostrongylus* sp. (myeloencephalitis)	Africa, America, Asia, Europe
Capillariasis	Africa, Asia
Dracunculiasis	Africa, America (central and south), Asia
Onchocerciasis	
Trematodes (blood flukes):	Africa, America (central and south), Asia
Schistosomiasis	
Liver flukes:	Worldwide
Fascioliasis	America (south), Asia, Europe
Opisthorchiasis	
Intestinal flukes:	Southern and Eastern Asia

Fasciolopsiasis	
Lung flukes:	
Paragonimiasis/ *Paragonimus* species	Far East, Indian subcontinent, Africa, some Pacific Isles
Cestodes (Tapeworms):	
Diphyllobothrium latum	America (north), Canada, Europe, Japan, Russia, Scandinavia
Hymenolepsis nana, Taeniasis/ *Taenia saginata/solium*, Cystercercosis/ *Taenia solium*	Worldwide
Echinococcosis/ *Echinococcus* sp.	Africa, America, Asia, Europe, Australasia
Sparganosis/ *Spirometra mansonoides*	America, Asia (particularly China and Japan)
Coenurosis/ *Taenia multiceps/serialis*	Africa, America, Europe

Appendix 3: Calibrating the microscope for measurement

Calibrating the microscope for measurement

The size of ova and cysts is an important identifying feature of many parasites.

Size can be determined with an eyepiece micrometer. The calibration of the microscope is carried out as follows:

Equipment required includes an eyepiece micrometer and a stage micrometer.

1. The eyepiece scale is divided in 100 small divisions.
2. The stage micrometer scale extends over 1mm being divided in 0.1mm divisions, each being divided again in 0.01mm divisions.
3. Insert the eyepiece graticule to the eyepiece, and replace into the microscope.
4. Place the stage micrometer on the microscope stage.
5. Focus the low-power objective on the stage scale.
6. Adjust the eyepiece and stage scales until they are parallel and overlap.
7. Note the number of eyepiece divisions and its corresponding stage measurement, for example, 10 eyepiece divisions = 0.20mm on the stage scale.
8. Calculate value of one eyepiece division as follows:

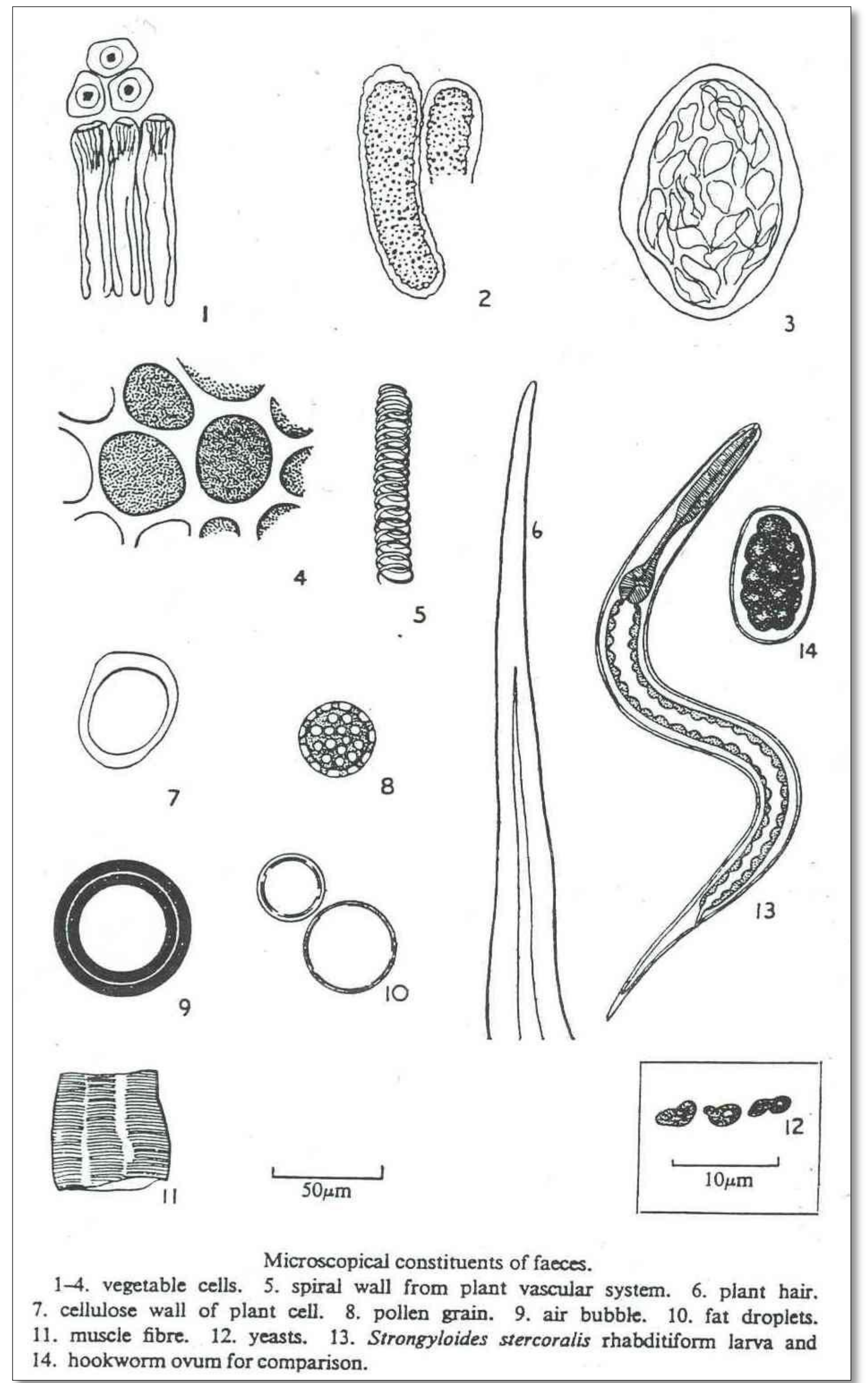
   10 eyepiece divisions = 0.20

   1 eyepiece division = 0.20/10 = 0.020mm = 20µm

   Note: To convert the calculated value (0.020mm) above to µm, it should be multiplied by 1000 µm/mm to give 20µm
9. Repeat from step 5 with each objective, noting and recording the reading from each.
10. Calibration need only be done once for each microscope, and its objectives and eyepieces.

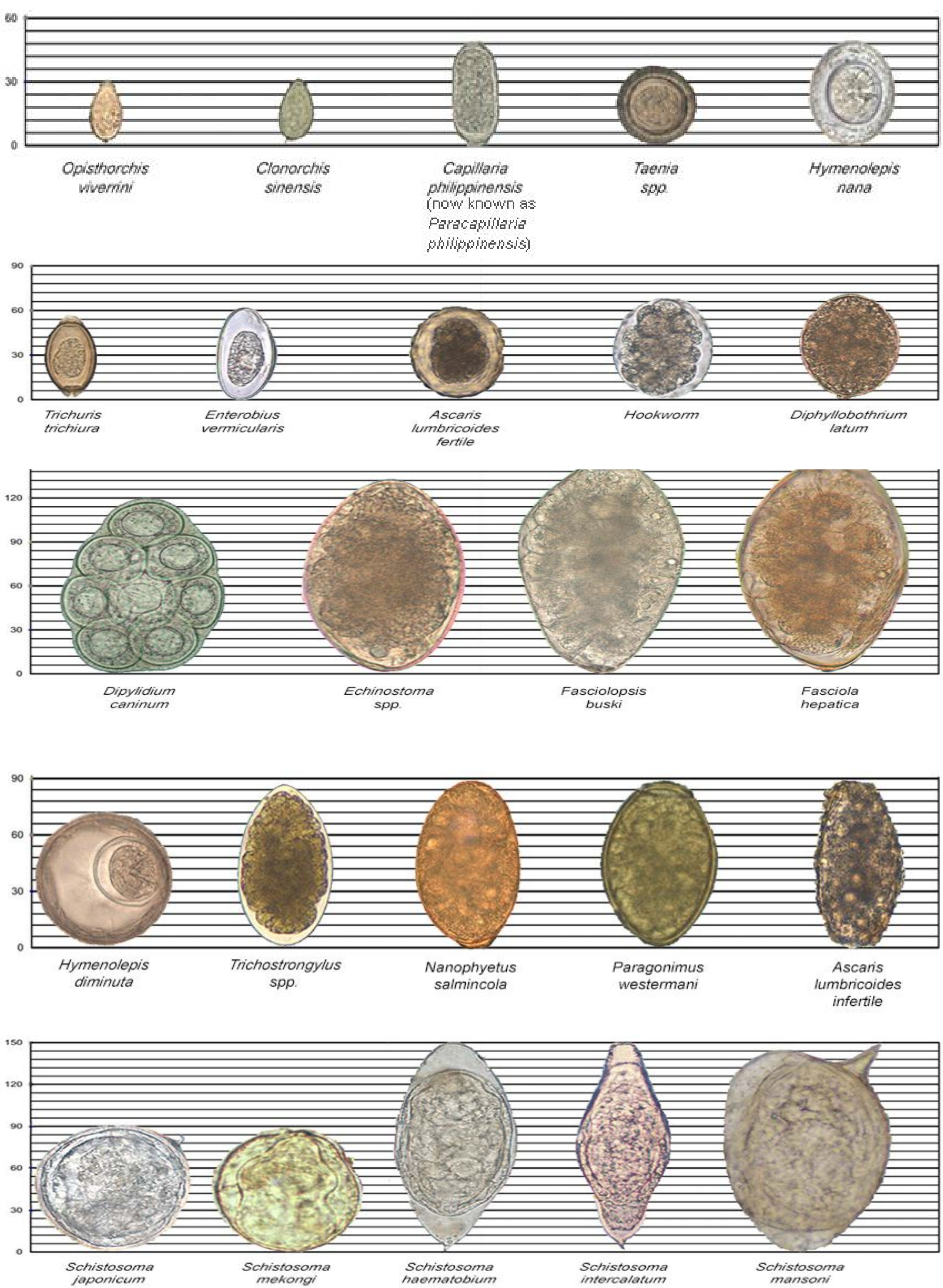
Courtesy of UK NEQAS Parasitology Teaching Programme 2003/2004; Faecal Parasites. Co-ordinated by Hilary Edwards.

Appendix 4: Common microscopic constituents of faeces[110]



Microscopical constituents of faeces.
1–4. vegetable cells. 5. spiral wall from plant vascular system. 6. plant hair. 7. cellulose wall of plant cell. 8. pollen grain. 9. air bubble. 10. fat droplets. 11. muscle fibre. 12. yeasts. 13. *Strongyloides stercoralis* rhabditiform larva and 14. hookworm ovum for comparison.

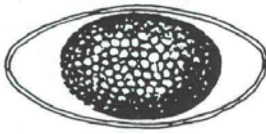
Appendix 5: Relative sizes of helminth eggs*

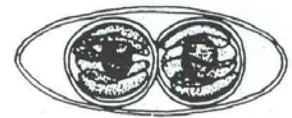


*Measurements in micrometres (µm)

Appendix 6: Oocysts of coccidia/ cryptogregaria/ microsporidia

***Cystoisospora belli*[1]**





Immature oocyst (32 x 16μm) Mature oocyst

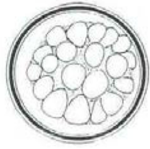
Oocysts are transparent. Reduced illumination is recommended. Modified Ziehl-Neelsen can be used for direct smears

***Cryptosporidium* species**

5μm

Auramine-phenol and modified Ziehl-Neelsen stains are recommended

***Cyclospora* species** (CLB)

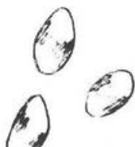


8 – 10μm

unsporulated sporulated

In an unstained wet preparation, a central morula contains several refractile spheres. In fresh water, the morula divides into 2 smaller structures. *Cyclospora cayetanesis* can be seen in formol-ether concentrations as refractile spheres which do not stain with iodine or auramine-phenol stain but are variably acid-fast, staining pink or not at all with modified Ziehl-Neelsen stain. It will auto-fluoresce blue at 340 – 360 nm

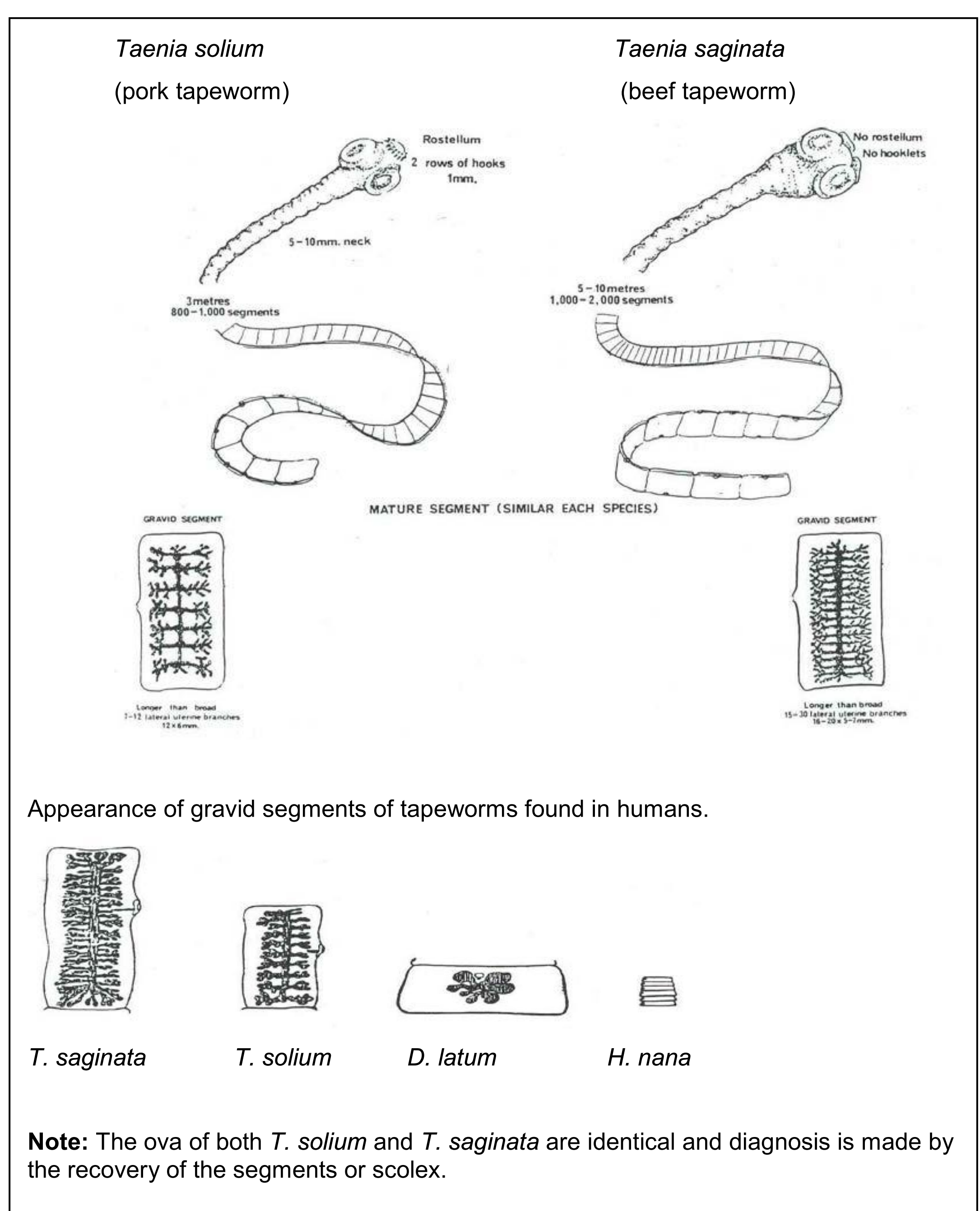
Microsporidia species



1-4μm depending on the species

Although microsporidia may appear acid-fast when stained with modified Ziehl-Neelsen, the trichrome stain is recommended. Microsporidial spores are ovoid and refractile and the spore wall stains bright pink-red. Occasionally the spores stain with a red `belt' across the centre of the spore, or show polar granules, which are both diagnostic features

Appendix 7: Comparison of tapeworms commonly found in humans[111]



Appearance of gravid segments of tapeworms found in humans.

T. saginata *T. solium* *D. latum* *H. nana*

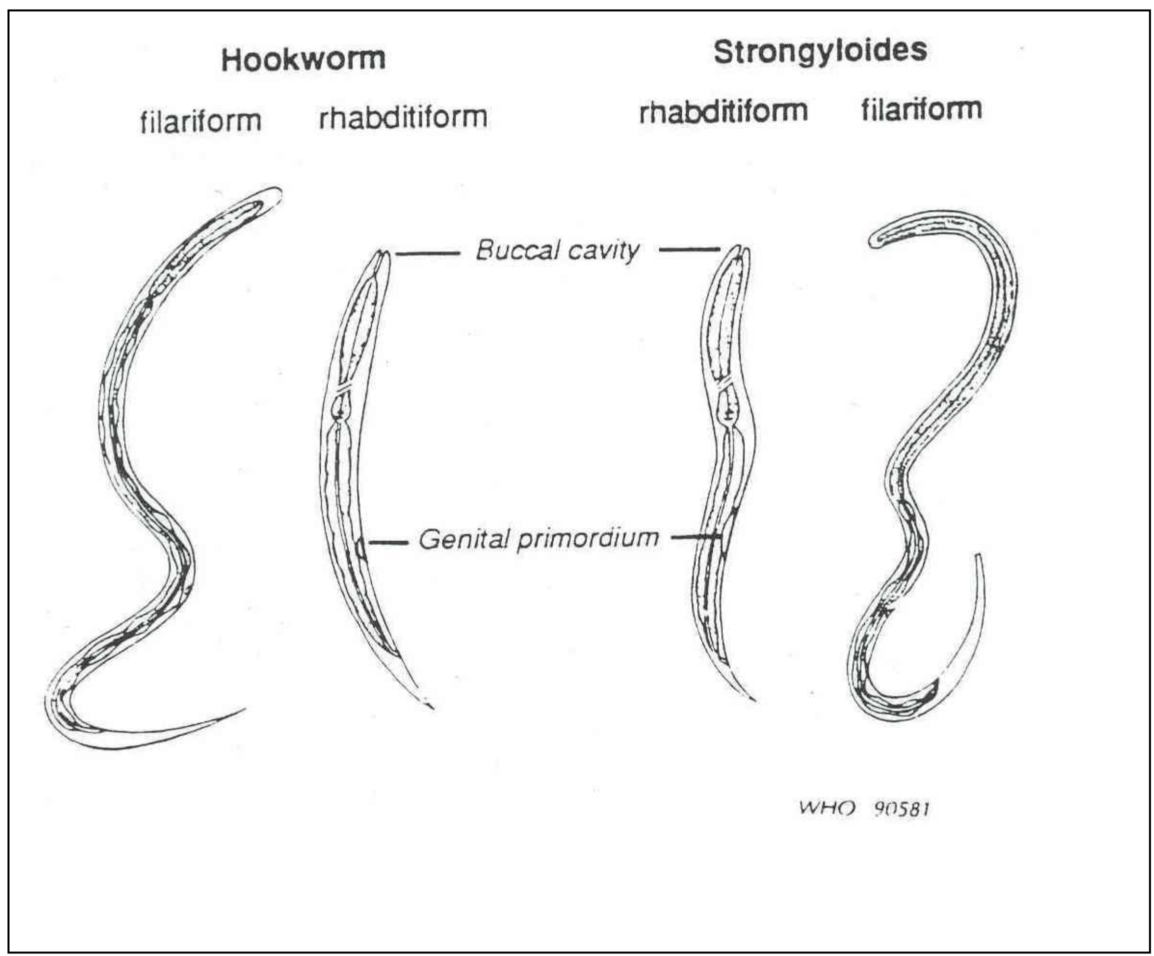
Note: The ova of both *T. solium* and *T. saginata* are identical and diagnosis is made by the recovery of the segments or scolex.

Appendix 8: Helminth larvae – characteristics[112]

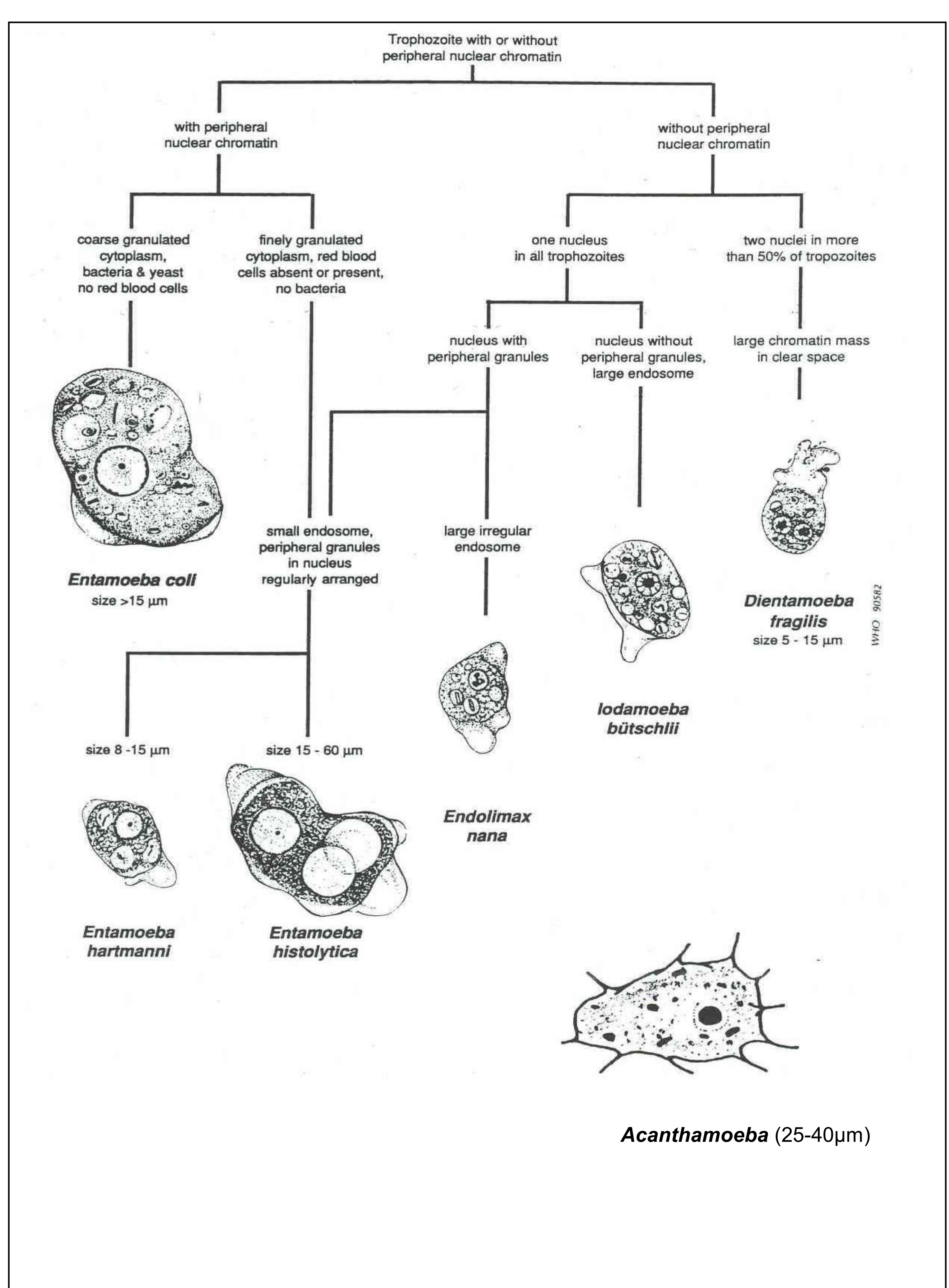
Hookworm	*Strongyloides*
Filariform larvae Size 500 x 14-20 µm Sheathed Tapered tail Oesophagus one-third of body length	*Filariform larvae* Size 500 x 14-20µm Unsheathed Blunt or forked tail Oesophagus half of body length
Rhabditiform larvae Size 100 - 150 x 15-17µm Long buccal cavity – 15µm Oesophagus one-third of body length with two swellings Genital primordium small – 7µm Anal pore 80µm from posterior end	*Rhabditiform larvae* Size 200 - 300 x 15-18µm Short buccal cavity – 4µm Oesophagus one-third of body length with two swellings Genital primordium large – 22µm Anal pore 50µm from posterior end

In fresh stool specimens, the most likely larvae to be seen are rhabditiform larvae of *Strongyloides stercoralis.* If the stool is >12 hours old, the larvae may develop into filariform larvae which must be differentiated from hookworm larvae (these may appear in the stool within 12–24hr).

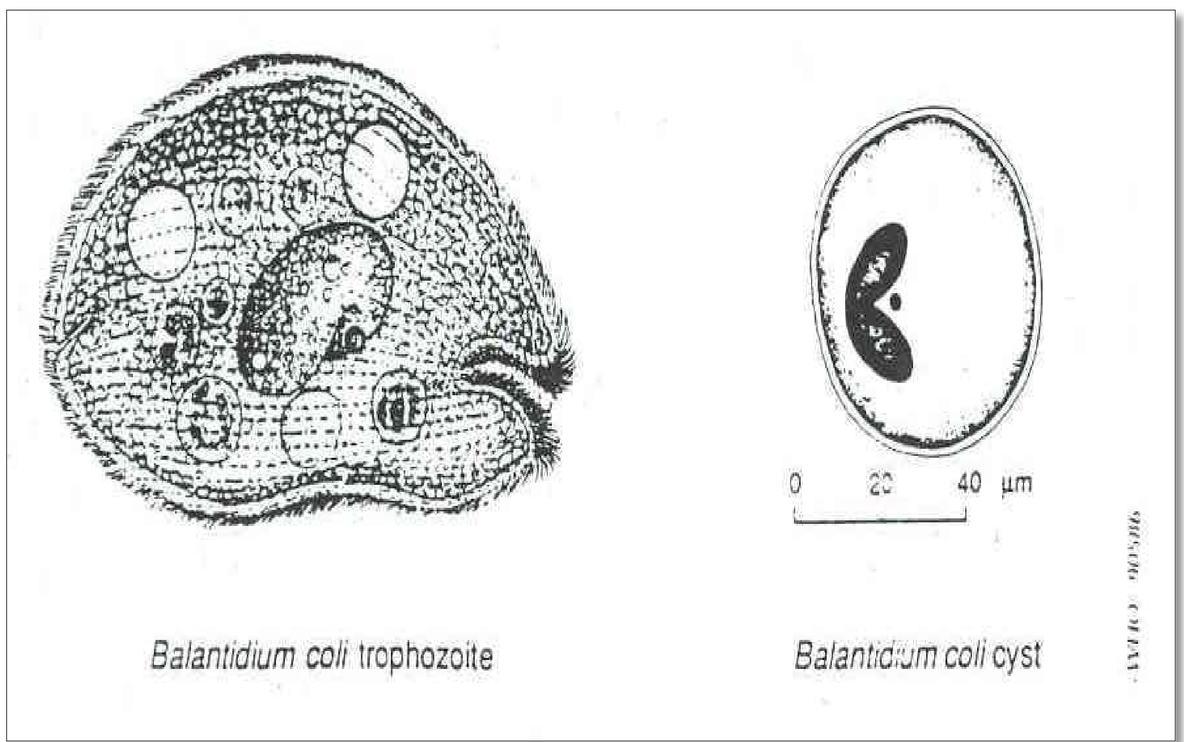
Helminth larvae



Appendix 9: Identification of amoebic trophozoites in stained smears[1]



Appendix 10: *Balantidium coli* - trophozoite and cyst[1]



The characteristics between the *Balantidium coli* Trophozoite and *Balantidium coli* cyst

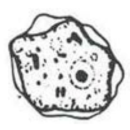
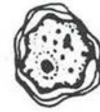
Trophozoite	Cyst
Oval and covered in short cilia measure approximately 30-150µm in length x 25-120µm in width but may attain lengths of up to 200µm A funnel shaped cytosome can be seen near the anterior end Micronucleus and macronucleus may be observed in stained preparations	Spherical or ellipsoidal Measures from 30-200µm by 20-120µm Contains 1 macronucleus and 1 micronucleus Presence of cilia in young cysts but disappear after prolonged encystment

Note: Wet preparations of fresh and concentrated stool samples reveal the characteristic cysts and motile trophozoites. They are easier to identify in direct-smear saline preparations than permanently stained faecal smears.

Appendix 11: Identification of amoebic and flagellate cysts

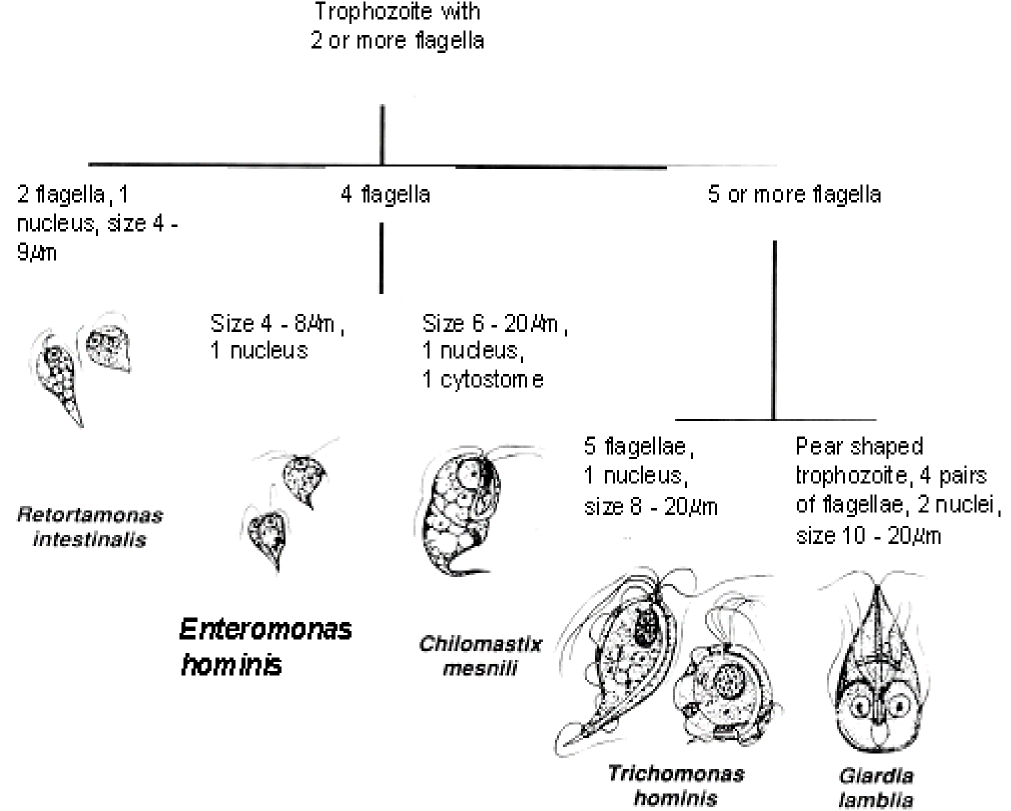
Cyst

- with filaments inside flagellate cyst
  - Pear-shaped, 1 nucleus, Size 4 - 7 µm — ***Retortamonas intestinalis***
  - Lemon-shaped, 1 nucleus, size 5 - 9µm — ***Chilomastix mesnili***
  - Oval cyst, Thick wall
    - Size 4 - 10µm, 1 to 4 nuclei — ***Enteromonas hominis***
    - Size 10 - 14µm, Parabasal bodies, 2 - 4 bodies piled at one end — ***Giardia lamblia***
- without filaments, amoebic cyst
  - Peripheral nuclear chromatin absent
    - 4 nuclei, large chromatin masses — ***Endolimax nana***
    - Large eccentric nucleus, iodine staining vacuole — ***Iodamoeba butschlii***
  - Peripheral nuclear chromatin present
    - < 10µm — 1, 2 or 4 nuclei, glycogen mass — ***Entamoeba hartmanni*** (immature cyst)
    - 10 –15µm
      - 1, 2 or 4 nuclei, glycogen mass (immature cyst)
      - 2,4 or 5 nuclei, even peripheral chromatin (mature cyst)
      - **Entamoeba histolytica / dispar**
    - 15 - 30µm — ***Entamoeba coli*** (Mature & immature cyst)

Acanthamoeba (11-30µm)

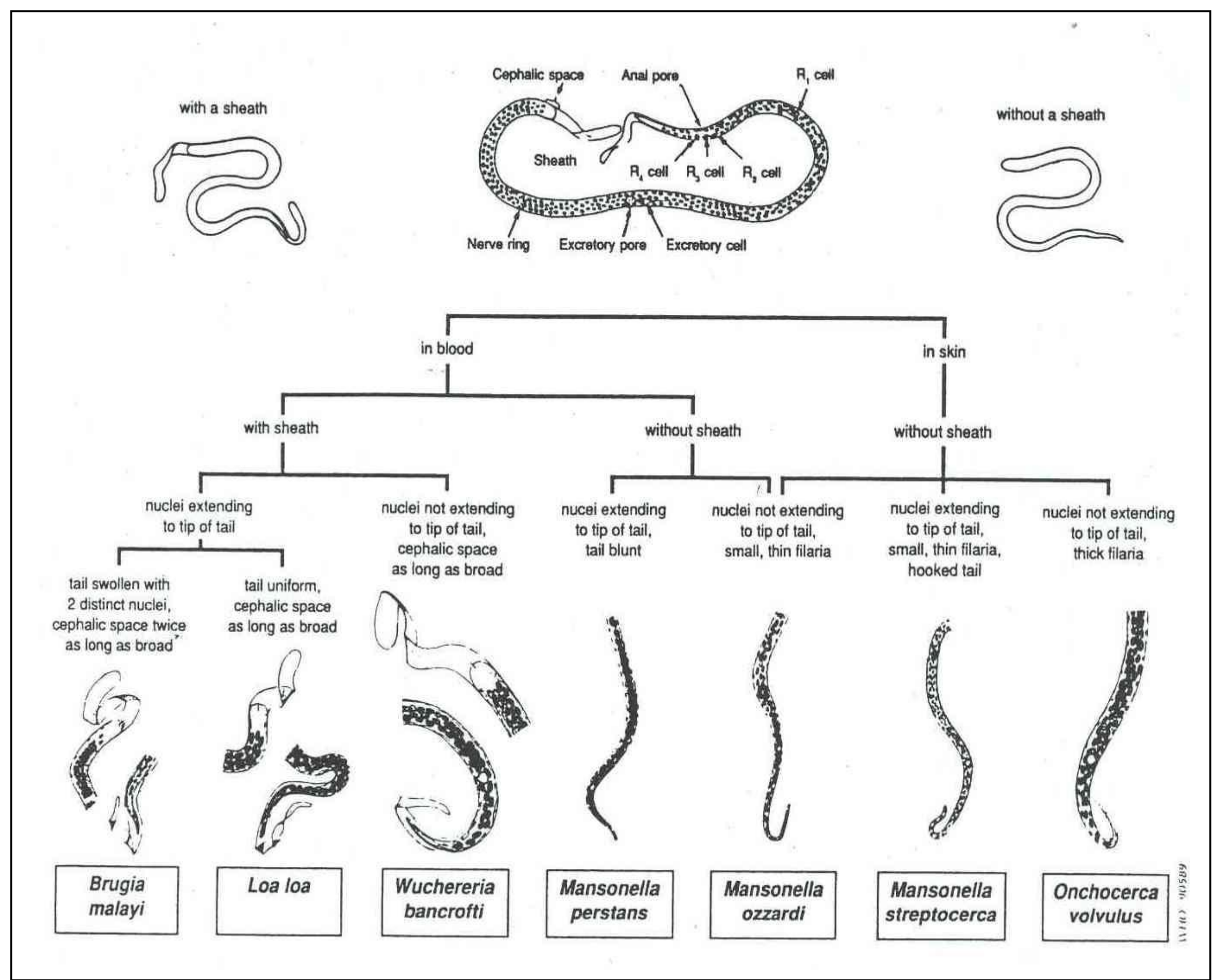
Appendix 12: Identification of flagellate trophozoites[1]



Note: It should be noted that *Trichomonas hominis* does not have a cyst stage.

(Adapted and redrawn, WHO, 1991)

Appendix 13: Microfilariae found in humans[1]



References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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14. Hobbs MM, Sena, A. C. Modern diagnosis of Trichomonas vaginalis infection. Sexually transmitted infections 2013;89:434-8. **B, III**

15. Association of Public Health Laboratories. Advances in Laboratory Detection of *Trichomonas vaginalis.* APHL. 1-5. 2013. **B, III**

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17. Lopez AS, Bendik JM, Alliance JY, Roberts JM, da Silva AJ, Moura IN et al. Epidemiology of Cyclospora cayetanensis and other intestinal parasites in a community in Haiti. JClinMicrobiol 2003;41:2047-54. **B, II**

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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 31 Investigation of specimens other than blood for parasites

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 21/09/2016 – 03/10/2016

Version of document consulted on: B 31dzm+

Proposal for changes

<table>
<tr><td>Comment number</td><td>1</td><td colspan="2"></td></tr>
<tr><td>Date received</td><td>29/09/2016</td><td>Lab name</td><td>Scottish Parasite Diagnostic and Reference Laboratory</td></tr>
<tr><td>Section</td><td colspan="3">a. page 10, Heading Protozoa , subheading Intestinal amoeba
b. Page 10, Heading Protozoa subheading Free-living amoebae
c. page 11 Flagellates
d. page 12 Trichomonas vaginalis
e. Page 12 Cyclospora
f. page 19 Schistosoma</td></tr>
<tr><td colspan="4">Comment</td></tr>
<tr><td colspan="4">a. Reporting cysts as E. histolytica / E. dispar causes confusion for users resulting in a number of queries asking for the results to be explained. Instead, as many laboratories detect these organisms using microscopy, they should perform antigen or molecular detection locally, or be advised to forward to appropriate specialist centres for these tests if these cysts are present by microscopy to distinguish between them.
b. (i) A national molecular service is now used for amoebic detection in most Scottish health boards.
(ii) No mention of corneal tissue (scrapings) being used for Acanthamoeba detection.
c. After a recent audit in Scotland, most laboratories use microscopy, not EIA for Giardia. Where possible, perform microscopy using both a concentrated and an unconcentrated sample as the number of cysts can be significantly reduced in certain samples post-concentration.
d. Molecular testing also available for TV.
e. Worth stating that recent outbreaks in UK travellers have been associated with travel to Mexico - this will ensure laboratory staff know to search for this pathogen in samples stating a Mexico travel history.
f. In Scotland, laboratory diagnosis is made by first testing sera for the presence of antibodies taken 8 weeks after last exposure to fresh water. Only if positive are faeces, urine or semen requested.</td></tr>
<tr><td colspan="4">Evidence</td></tr>
<tr><td colspan="4">https://www.gov.uk/government/news/cyclospora-outbreak-linked-to-mexico</td></tr>
</table>

Health benefits	
Treatment would be prevented if laboratories reported *E. dispar* instead of *E. histolytica* / *E.dispar* using appropriate tests to distinguish between the two.	
Recommended action	a. **ACCEPT** This has been updated accordingly. b. (i) **NONE** This has already been added in the document. (ii) **NONE** The information on corneal tissue (scrapings) being used for *Acanthamoeba* detection has been moved into the SMI B 2: Investigation of bacterial eye infections document. c. **ACCEPT** This has been updated accordingly. d. **ACCEPT** This has been updated accordingly. e. **ACCEPT** This section will be redrafted and updated accordingly. f. **NONE** Not relevant to all samples.

Comment number	2		
Date received	03/10/2016	**Lab name**	Cryptosporidium Reference Unit
Section	a. P12 b. P12 c. P13 d. P13 e. P22 f. Section 4.1 g. Appendix 2 h. Appendix 3		
Comment			
a. Following extensive review of molecular and biological data, *Cryptosporidium* has been formally transferred from the Coccidia, to a new subclass with gregarine parasites, Cryptogregaria. b. The slash needs to be removed between microscopy and stains in the following			

sentence. Primary laboratory diagnosis is based on antigen detection by enzyme immunoassay followed by confirmation using microscopy/stains or DNA detection by PCR. c. This is not an accurate picture and needs to be re-written: *Cyclospora* infection occurs in many countries and may be associated with drinking or bathing in contaminated water. Large outbreaks affecting travellers and foreign residents have being known to occur during the rainy season in South Asia and North America[15,16]. Please contact me if you want me to help re-write it! d. This gives the impression it is routine. It is not and there are difficulties. Whole genome sequencing has been used to detect and subtype *Cyclospora cayetanensis* especially in outbreak investigations[20]. There are multi-locus schemes too but not formal ones. e. Outdated wording in this sentence - use in humans or people not man! Cysticercosis (this is tissue infection with cysticerci of *T. solium*) can develop in man by autoinfection from the adult worm. Involvement of the central nervous system is called neurocysticercosis[54]. f. All faecal samples from symptomatic individuals should be stained for *Cryptosporidium* oocysts[81]. Replace stained with tested. g. Columns are out of alignment. When it says worldwide why is United States also mentioned? Isn't this included in worldwide? h. Appendix 6: Oocysts of coccidian, not coccidian.
Evidence
a. Cavalier-Smith, T., 2014. Gregarine site-heterogeneous 18S rDNA trees, revision of gregarine higher classification, and the evolutionary diversification of Sporozoa. Eur. J. Protistol. 50 (5), 472e495. b. Chalmers RM, Atchison C, Barlow K, Young Y, Roche A, Manuel R. An audit of the laboratory diagnosis of cryptosporidiosis in England and Wales. Journal of Medical Microbiology 2015 64: 688-693 c. Nichols GL, Freedman J, Pollock KG, Rumble C, Chalmers RM, Chiodini P, Hawkins G, Alexander CL, Godbole G, Williams C, Kirkbride HA, Hamel M, Hawker JI. Cyclospora infection linked to travel to Mexico, June to September 2015. Euro Surveill. 2015;20(43):pii=30048. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.43.30048

Recommended action	a. **ACCEPT** This has been updated accordingly. b. **ACCEPT** The slash has been removed and a comma added instead. c. **ACCEPT** This section will be redrafted and updated accordingly. d. **ACCEPT** This has been rephrased to acknowledge that routine laboratories do not currently use this cutting edge

	technique. e. **ACCEPT** This has been updated accordingly. f. **ACCEPT** This has been updated accordingly. g. **ACCEPT** The columns have been realigned and updated accordingly. United States have also been removed. h. **ACCEPT** This has been updated accordingly. This reference (by *Chalmers RM, Atchison C, Barlow K, Young Y, Roche A, Manuel R. An audit of the laboratory diagnosis of cryptosporidiosis in England and Wales. Journal of medical microbiology 2015; 64:688-93*) is already in the document. The other two references mentioned above (a and c) have been accepted and added within the document where appropriate.

Comments received outside of consultation

Comment number	1		
Date received	07/10/2016	**Lab name**	Royal Cornwall Hospitals Trust
Section	All		
Comment			
We have no formal comments, apart from formatting and grammatical comments, which I assume you will pick up (for example, pp13 the *Sarcocystis* paragraph mentions eating undercooked meat or infected cats).			
Recommended action	**ACCEPT** This has been amended and updated accordingly.		

Comment number	2		
Date received	13/10/2016	**Lab name**	Reference Microbiology Services
Section	All		
Comment			

Reference Microbiology Services have sent track changes for this document.	
Recommended action	**ACCEPT** The changes have been accepted and updated accordingly where necessary.

Comment number	3		
Date received	13/10/2016	**Lab name**	Hospital for Tropical Diseases and PHE National Parasitology Reference Laboratory
Section	All		
Comment			
HTD and PHE National Parasitology Reference Laboratory have sent track changes for this document.			
Recommended action	**ACCEPT** The changes have been accepted and updated accordingly where necessary.		

Respondents indicating they were happy with the contents of the document

Overall number of comments: 4			
Date received	03/10/2016	**Lab name**	Member of the public
Date received	03/10/2016	**Lab name**	Member of the public
Date received	03/10/2016	**Lab name**	Member of the public
Date received	03/10/2016	**Professional body**	Healthcare Infection Society

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Investigation of bone marrow

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 38 | Issue no: 2 | Issue date: 12.10.15 | Page: 1 of 22

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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 11

2 SPECIMEN COLLECTION 11

3 SPECIMEN TRANSPORT AND STORAGE 12

4 SPECIMEN PROCESSING/PROCEDURE 12

5 REPORTING PROCEDURE 15

6 NOTIFICATION TO PHE, OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 16

APPENDIX 1: INVESTIGATION OF BONE MARROW BY CULTURE 18

REFERENCES 19

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	3/12.10.15
Issue no. discarded.	1.2
Insert issue no.	2
Section(s) involved	**Amendment**
Page 2.	Updated logos added.
Scope.	Text updated for clarity.
Introduction.	Reorganised for clarity. Rapid methods section added.
Technical information/limitations.	Addition of section on anticoagulants.
Safety considerations.	Reviewed and updated. If Hazard Group 3 organisms are not suspected, consider processing under Containment Level 2 conditions. Information added regarding thermally dimorphic fungi.
Specimen collection.	Use of blood culture bottles recommended. Additional specimens for direct culture, microscopy and molecular techniques should be collected in appropriate CE marked leak proof containers.
Specimen processing/procedure.	Addition of direct culture and molecular techniques. Section 4.5.1 culture media, conditions and organisms updated. Incubate blood culture broths for 5 days. Incubate FAA for 5 days. Incubate Sabouraud agar for 14 days.
Reporting procedure.	Addition of reporting for molecular methods.
Appendix 1.	Updated in line with section 4.5.1.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

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Scope of document

Type of specimen

Bone marrow

This SMI describes the processing and microbiological investigation of bone marrow samples submitted for clinical diagnostic purposes. Techniques covered by this SMI include culture of bone marrow for the identification of bacteria and fungi, as well as molecular methods and rapid techniques. Other methods of investigation are available for the identification of parasites and viruses, but are not covered in this SMI.

For the investigation of bone marrow for *Mycobacterium* species refer to B 40 – Investigation of specimens for *Mycobacterium* species.

This SMI should be used in conjunction with other SMIs.

Introduction

Microbiological examination of bone marrow is an invasive technique infrequently performed for the investigation of pyrexia of unknown origin (PUO) and occasionally for other indications[1]. It is sometimes undertaken when other less invasive investigations and diagnostic imaging have failed to determine a cause, or, more frequently, when infection is part of the differential diagnosis in the investigation of haematological abnormalities[2]. The demonstration of microorganisms in bone marrow by microscopy, culture or nucleic acid amplification techniques is useful for diagnosis of infection with a limited number of bacteria, fungi, parasites and viruses[3-5].

Bone marrow is aspirated from the posterior iliac crest or the sternum; a core biopsy may also be collected, and this can be examined histologically for evidence of granulomata and microorganisms. The aspirate is however the preferred specimen for microbiological studies.

Infection in patients who are immunocompromised

It has been suggested that bone marrow cultures should not be used for immunocompetent patients, but should be reserved for patients who are severely immunosuppressed[6]. Conditions leading to significant immunosuppression such as advanced HIV infection, bone marrow or solid organ transplant, or high dose corticosteroid therapy predispose patients to infection with opportunistic pathogens and make disseminated infection with pathogens more likely[7]. In these cases culture of bone marrow may be useful in the investigation of pyrexia of unknown origin (PUO)[2,8-10]. *Mycobacterium* species, *Histoplasma capsulatum, Paracoccidioides brasiliensis, Talaromyces marneffei* (formerly *Penicillium marneffei*) *and Leishmania* species are likely to cause disseminated infection in the setting of immunosuppression[6,11].

Organisms which have been demonstrated in bone marrow

Some organisms invade bone marrow as part of a multi-system infection, whereas others have a tropism for bone marrow or the cell lines therein. In several studies, culture of bone marrow has been shown to be a faster and more sensitive method of isolation of certain organisms (for example *Brucella* species and *Salmonella* Typhi) compared to blood culture. However, in some studies similar yields and turnaround

times were observed[5,12-14]. Bone marrow cultures may be positive in patients with acute or chronic infection, whereas blood cultures are more likely to be positive in patients with acute infections[13]. Bone marrow aspirates are also more likely than blood culture to be positive in patients who have been treated with antibiotics[5,15].

Bone marrow examination is most likely to be performed for the organisms below. The list is not exhaustive; other organisms may be detected or isolated.

Bacteria

Salmonella Typhi and *Salmonella* Paratyphi

Salmonella Typhi and *Salmonella* Paratyphi (groups A, B, and C*)* are the causative organisms of enteric (typhoid) fever and are usually carried by humans, and transmitted via contaminated food or water[5]. Enteric fever is the only bacterial infection for which bone marrow is routinely recommended[16]. Culture of bone marrow is considered to be the 'gold standard' method for diagnosis of typhoid fever. Blood culture may lack sensitivity and culture of bone marrow aspirates has been shown to produce a higher yield even when following antimicrobial treatment[5,16]. In one study it was shown that 1mL of bone marrow gave an equivalent result to 15mL of blood[17]. Serology is available, but has low sensitivity and specificity due to cross reactions with other *Salmonella* species and Enterobacteriaceae[17]. Nucleic acid amplification tests (NAATs) on culture positive bone marrow aspirates have been reported, but are not yet in routine use[18]. Cultures of *S.* Typhi and of *S.* Paratyphi A, B or C (known or suspected) must be handled at Containment Level 3.

Brucella species

Brucella is a zoonotic disease which has a wide range of symptoms and is thought to be greatly under diagnosed. Laboratory diagnostic techniques include culture, NAATs and antibody detection (the presence of antibodies is not always indicative of active brucellosis). Recovery from blood is suboptimal and it has been suggested that culture of bone marrow (as well as liver tissue and lymph nodes) may improve the recovery rate within a shorter time frame[12,13,19].

Mycobacterium species

Mycobacterium species are considered an important cause of pyrexia of unknown origin. Tuberculosis is primarily caused by *Mycobacterium tuberculosis.* A number of non-tuberculous mycobacterial species have been isolated from systemic infections in patients who are HIV positive. Culture is considered the 'gold standard' method for laboratory diagnosis, however incubation times may be long[3]. The use of continuous blood culturing systems reduces culture time; positive results may be available within five to seven days[19]. Bone marrow culture assists in aiding diagnosis in uncertain cases of disseminated disease, particularly in those with HIV[14,20-22]. Molecular methods for detection are currently under development (refer to B 40 – Investigation of specimens for *Mycobacterium* species)[3].

Fungi

Infection with dimorphic fungi such as *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* or *Talaromyces marneffei* (formerly *Penicillium marneffei*) may occasionally be diagnosed by bone marrow examination, but culture sensitivity varies[11,23]. Culture for *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* may take between two and six weeks; continuous monitoring blood culture systems have been shown to reduce culture time of *Talaromyces marneffei* to about four days[24-26]. It

has been suggested that culture of bone marrow samples may be more sensitive than other tests; however, diagnosis is more frequently made by detection of these organisms in respiratory and tissue specimens[14].

Parasites

Leishmania species

There are over 20 species of the protozoan parasite *Leishmania.* Humans are infected by the bite of infected female sandflies. The disease is endemic in five continents and over eighty countries. Leishmaniasis presents as three distinct syndromes, visceral (also known as Kala-azar), cutaneous and mucosal. Visceral Leishmaniasis, for which bone marrow investigation may be performed, can be fatal if untreated and is characterised by fever, weight loss, hepatosplenomegaly and pancytopenia[27]. Co-infection with HIV in endemic areas is associated with a more rapid progression to AIDS and infection has been transmitted through needle-sharing by infected drug users in south west Europe[1].

Following presumptive identification using Giemsa stain to detect amastigotes, samples should be sent to the reference laboratory for confirmation. Rapid diagnostic tests including direct agglutination and immunochromographic tests (ICT) have been developed and evaluated[1,28]. Serological diagnosis is available but it is significantly less sensitive in those with advanced HIV coinfection than for HIV negative individuals. Negative results should not therefore be used to rule out a diagnosis in those with HIV[4,27]. Cross-reactions can occur in patients with prior exposure to *Trypanosoma cruzi*. Splenic puncture is the most sensitive test, but bone marrow examination is safer and has a sensitivity of around 70 – 80%[1,27].

Viruses

Many viruses can be detected in bone marrow samples. Viral detection indicates infection, but does not necessarily confirm diagnosis of disease. The clinical significance of a positive bone marrow result is dependent on the immune status of the patient and the disease/illness under investigation; positive results from bone marrow samples must therefore be interpreted with caution. Routinely, NAATs or serology on peripheral blood is used for diagnosis of acute viral infection. In the immunocompromised, blood serology results may be negative at the onset of clinical disease. If there is a high clinical suspicion of viral infection, but peripheral blood NAATs results are negative, diagnosis may be confirmed by bone marrow examination.

Rapid techniques

Molecular methods[29-31]

NAAT - Nucleic Acid Amplification Techniques (eg PCR) for the identification of bacteria, fungi, parasites and viruses from clinical samples have been shown to be highly specific and sensitive. PCR targets conserved genes of the genome, and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short timeframe particularly if multiplex real-time PCR is used.

MALDI-TOF mass spectrometry[32,33]

Recent developments in identification of bacteria and yeast include the use of 16s ribosomal protein profiles obtained by Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) mass spectrometry. Mass peaks achieved by the test strains are compared to those of known reference strains. It is possible for an organism to be identified from an isolate within a short time frame and it is increasingly being used in laboratories to provide a robust, rapid and effective identification system for bacterial and yeast isolates.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers[34,35]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Anticoagulants[36]

Specimens for direct culture, microscopy and molecular techniques should be collected in appropriate CE marked leak-proof containers. Various tubes containing anticoagulants may be used, manufacturer's instructions should be consulted prior to use.

Specimens for direct culture and microscopy may be submitted in a plain sterile tube, or a sterile heparinised tube. Specimens for NAAT may be submitted in sterile tubes containing heparin or EDTA.

1 Safety considerations[34,35,37-51]

1.1 Specimen collection transport and storage[34,35,37-40]

Use aseptic technique.

Ideally, specimens for culture should be collected directly into blood culture bottles and transported in sealed plastic bags.

Additional bone marrow specimens should be submitted in an appropriate CE marked leak-proof containers and transported in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[34,35,37-51]

Where Hazard Group 3 organisms (eg *Mycobacterium tuberculosis, Salmonella* Typhi, *Salmonella* Paratyphi, dimorphic fungi and *Brucella* species) are suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

If Hazard Group 3 organisms are not suspected, consider processing under Containment Level 2 conditions.

All laboratory procedures (including the examination of plates and cultures) must be conducted in a microbiological safety cabinet[43].

Some Hazard Group 3 fungi are thermally dimorphic, and will grow as yeast form in blood culture bottles and sub-cultures at 37°C, but as the highly infective mould form when sub-cultured onto agar plates incubated at 28-30°C. Care should be taken with yeast isolates if there is a relevant travel history, especially in HIV-infected individuals.

If blood culture bottles are employed to provide an enrichment broth then any consequential use and subsequent disposal of syringes and needles must comply with local safety protocols.

Specimen containers must also be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Bone marrow, bone marrow sample inoculated in a blood culture bottle

2.2 Optimal time and method of collection[52]

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[52].

Specimens for culture should ideally be collected in blood culture bottles.

Additional specimens for direct culture, microscopy and molecular techniques should be collected in appropriate CE marked leak-proof containers. For information regarding appropriate use of anticoagulants refer to technical information/limitations.

2.3 Adequate quantity and appropriate number of specimens[52]

As large a sample as possible should be obtained, with the caveat that volumes of >3mL are likely to be contaminated with peripheral blood which may have a dilution effect.

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

3 Specimen transport and storage[34,35]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[52].

4 Specimen processing/procedure[34,35]

4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species.

Refer to B 40 – Investigation of specimens for *Mycobacterium* species.

4.2 Appearance

N/A

4.3 Sample preparation

4.3.1 Pre-treatment

Standard

If not already done, inoculate blood culture bottles with specimen and load onto the automated continuous monitoring blood culture system. Subculture positive bottles as required (see B 37 – Investigation of blood cultures (for organisms other than *Mycobacterium* species)).

Optional

N/A

4.3.2 Specimen processing

Standard

Bottles that flag as positive on the automated system should be subcultured according to the same procedure as for blood culture bottles (see B 37 – Investigation of blood cultures (for organisms other than *Mycobacterium* species)).

Optional

Specimens collected into appropriate CE marked leak proof containers should be used for microscopy and may be used for the following tests:

Direct culture

Where clinically indicated, direct plate culture may be required. Refer to section 4.5.

Molecular techniques

Specimens for molecular testing should be processed according to manufacturer's instructions.

4.4 Microscopy

4.4.1 Standard

Giemsa stain

Giemsa stains should be carried out for Leishmaniasis as indicated by local protocols; a smear maybe made at the patient's bedside or at the receiving laboratory.

Refer to TP 39 – Staining procedures

4.4.2 Optional

Gram stain

Refer to TP 39 – Staining procedures

4.4.3 Supplementary

See B 40 - Investigation of specimens for *Mycobacterium* species, and TP 39 - Staining procedures

4.5 Culture and investigation

Inoculate each agar plate using a sterile pipette (Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
All clinical conditions	Bone marrow	Blood culture broths (aerobic and anaerobic) Subculture all bottles onto subculture plates below.	35 – 37	Air	5 d + terminal subculture	Continuous monitoring	Any organism
Subculture plates	Bone marrow	Blood agar	35 – 37	5 – 10% CO_2	40 – 48hr	≥40hr	Any organism
		Chocolate agar	35 - 37	5 – 10% CO_2	40 - 48hr*	≥40hr	Any organism
		FAA	35-37	Anaerobic	5 d	3 d and 5 d	Anaerobes

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Systemic fungal infection	Bone marrow	Sabouraud agar (slopes)	28 - 30	Air	14 d	Daily	Yeast and Mould
Where clinically indicated	Bone marrow	Direct Culture: Blood agar	35 – 37	5 – 10% CO_2	40 – 48hr	≥40hr	Any organism
		Chocolate agar	35 – 37	5 – 10% CO_2	40 - 48hr*	≥40hr	Any organism
		FAA	35-37	Anaerobic	5 d	3 d and 5 d	Anaerobes

Optional Molecular Techniques

Clinical details/ conditions	Specimen	Molecular Technique	Instructions	Target organism(s)
All clinical conditions	Bone marrow	NAAT	Follow manufacturer's instructions	Any organism

Other organisms for consideration - *Mycobacterium* species (see B 40 - Investigation of specimens for *Mycobacterium* species), fungi, parasites (see B 31 - Investigation of specimens other than blood for parasites) and viruses (see https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#virology).

*Incubation times may be increased up to 5 days if *Brucella* species infection is suspected.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

All organisms to species level.

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: Any organism considered to be a contaminant may not require identification to species level.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

5.1.1 Standard

Giemsa stain

Report as indicated by local protocols.

5.1.2 Optional

Gram stain

Report organism detected.

Supplementary

For the reporting of microscopy for *Mycobacterium* species refer to B 40 – Investigation of specimens for *Mycobacterium* species.

5.1.3 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

Following results should be reported:

- clinically significant organisms isolated
- other growth
- absence of growth

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

5.3 Molecular

Report results as per manufacturer's instructions.

5.4 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[53,54], or equivalent in the devolved administrations[55-58]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and

as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

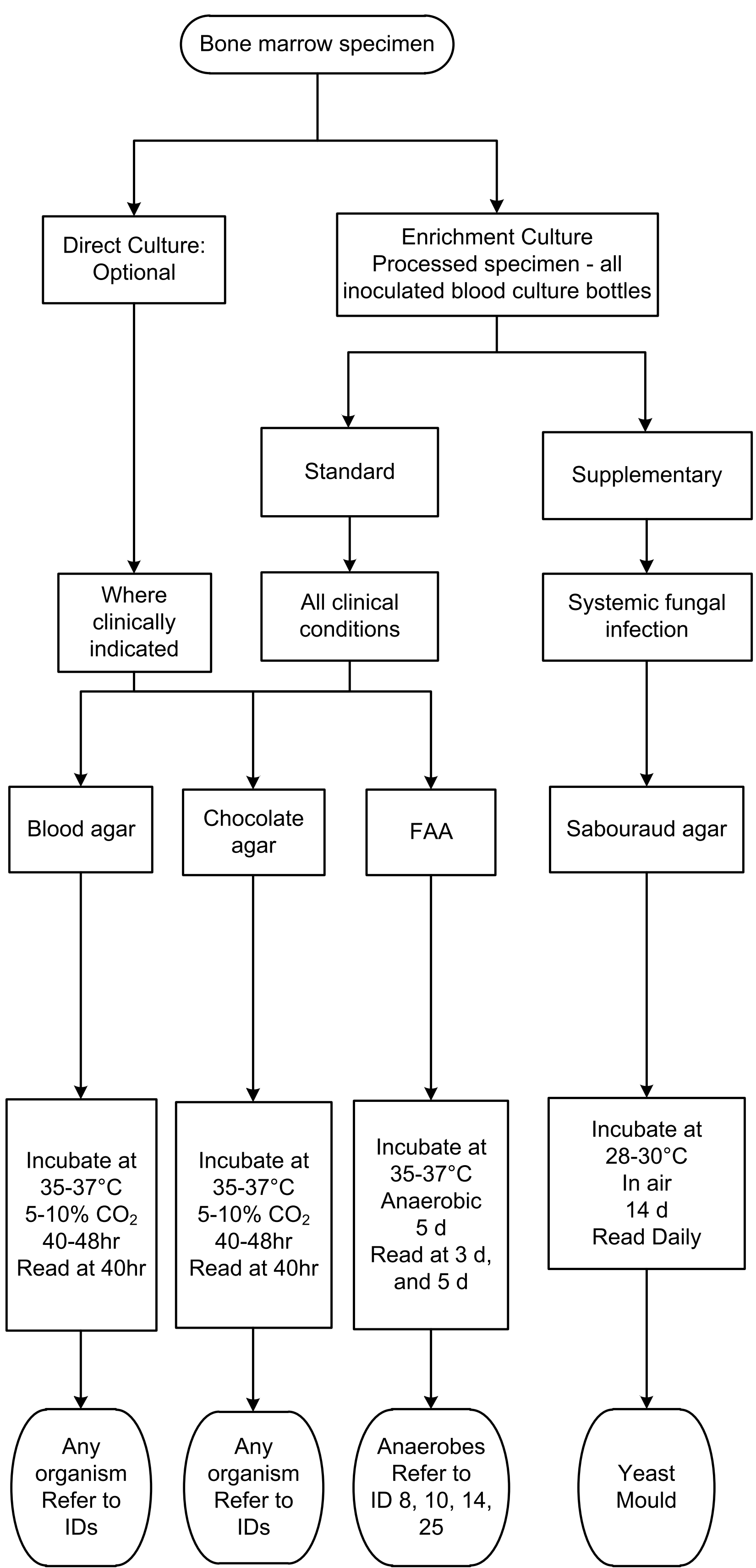
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[55,56], Wales[57] and Northern Ireland[58].

Appendix 1: Investigation of bone marrow by culture



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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working group for microbiology standards in clinical bacteriology

B 38 Investigation of bone marrow

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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A50711295

Consultation 15.09.14 – 13.10.14

Version of document consulted on – B 38dr+

Proposal for changes

Comment number	1		
Date received	10/10/2014	**Lab name**	Royal Liverpool University Hospital
Section	4.5 Culture and Investigation		
Comment			
a. We note the advice to use enrichment broth and examine the broth daily and subculture if positive. We felt this would be challenging as the broth Robinsons Cooked Meat Broth tends to become cloudy secondary to break down of the constituents of the broth, regardless of the presence of bacteria. b. We also note the suggestion to keep the FAA plate for 48 hours only - our practice is to extend the incubation to 5 days as our experience is that anaerobes are frequently isolated beyond 2 days. We would therefore be concerned regarding missing anaerobic growth, particularly as our empirical antibiotic treatment of bone infection does not always include anaerobic cover.			
Recommended action	**NONE** These comments are based on a previous version of the document, not the version of the document under consultation. Both points have been addressed.		

Comments received outside of consultations

Comment number	1		
Date received	01/07/2013	**Lab name**	MSTAG
Section	All 4 - 2.5.3		
Comment			
a. Blood culture broths - are both aerobic and anaerobic broths recommended? b. Incubation time 7-21d, conflicting advice regarding *Brucella* given in previous section, is this what is meant by "slow grower"? c. Microscopy suggesting anaerobes - not felt to be relevant to bone marrow, as cannot tell if anaerobes by microscopy. d. Neomycin - would this be needed, it was felt that FAA alone should suffice.			
Recommended action	a. **ACCEPT** Both bottles are recommended, this has been clarified in the text and table. b. **ACCEPT**		

	Brucella are expected in most cases to grow witin 5-7 days. The table has been updated in line with the other SMI documents to state a 5 day incubation. Reference to slow growers has been removed. c. **PARTIAL ACCEPT** It is accepted that it is not possible to differentiate anaerobes from aerobes by microscopy. FAA for anaerobes has been moved to the standard media section of the media table under subculture plates. Table and appendix updated. d. **ACCEPT** FAA rather than neomycin FAA with a metronidazole disc is recommended. Table and Appendix updated.

Comment number	2		
Date received	02/08/2013	**Lab name**	British Infection Association (BIA)
Section	Table 2.5.3 - incubation and culture of samples potentially containing anaerobes for microscopy.		
Comment			
Minor inconsistency between duration of incubation (7d) and reading (5d).			
Recommended action	**ACCEPT** Table reviewed and updated. Incubation FAA for 5 days, read at 3 and 5 days.		

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	29/09/2014	**Lab name**	Public Health Wales (PHW)

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Investigation of Dermatological Specimens for Superficial Mycoses

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 39 | Issue no: 3.1 | Issue date: 29.12.16 | Page: 1 of 26

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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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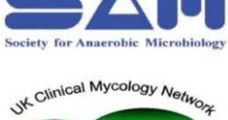

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Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

SCOPE 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 13

2 SPECIMEN COLLECTION 13

3 SPECIMEN TRANSPORT AND STORAGE 14

4 SPECIMEN PROCESSING/PROCEDURE 15

5 REPORTING PROCEDURE 21

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 23

REFERENCES 24

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	5/29.12.16
Issue no. discarded.	3
Insert Issue no.	3.1
Section(s) involved	**Amendment**
Scope.	Link to Doctor Fungus website removed.
5.2 Culture.	Spelling errors corrected.

Amendment No/Date.	4/11.08.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk. Taxonomy updated.
Page 2.	Updated logos added.
Technical information/limitations.	Microscopy updated.
Safety considerations.	Section 1.1 updated and expanded.
Specimen collection.	Clarification given throughout the section on how much sample to take.
Culture and investigation.	Clarification on the process for negative cultures has been included.
4.5.3.	PCR and Maldi Tof added as testing method.
References.	References reviewed and updated.

UK SMI#: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

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Scope of document

Type of Specimen

Skin, nail, hair

Scope

This SMI describes the procedures used to visualise and isolate dermatophytes, non-dermatophyte moulds and other fungi from skin, nail and hair specimens.

This SMI should be used in conjunction with other SMIs.

For descriptions and illustrations of structures observed on microscopy and/or culture refer to reference textbooks[1-4].

Introduction

Dermatophytes[5]

Dermatophytes can be divided into three groups[5]:

- anthropophilic dermatophytes are passed from human to human and are the most common in the community.
- zoophilic or animal acquired infections are usually sporadic.
- geophilic dermatophytes are most often acquired following a close association with soil or from an animal itself infected by soil contact.

Infection is usually diagnosed by observing the presence of fungal hyphae in skin, hair or nail specimens. However, it is important to culture the material to determine the infecting genus and species. This is done to ensure selection of the most appropriate therapy and in order to trace its likely epidemiology which may help in the management of infection.

Dermatophyte infections (commonly known as ringworm) are usually referred to as tinea followed by the Latin name of the body area involved. The most common dermatophyte infections are tinea pedis in adults (athlete's foot) which may also include tinea unguium (nail infection), and tinea capitis (scalp ringworm) in children.

Infection by dermatophytes is cutaneous and generally restricted to the non-living cornified layers in patients who are immunocompetent. This is because the dermatophyte group of fungi are generally unable to penetrate tissues which are not fully keratinised (ie deeper tissues and organs). However, reactions to such infections can range from mild to severe depending upon the host's immune response, the virulence of the infecting species, the site of infection and environmental factors.

The dermatophyte fungi are classified in three genera: *Epidermophyton* species, *Microsporum* species and *Trichophyton* species.

Non-dermatophytes

There are few non-dermatophyte moulds that can infect otherwise healthy skin and these include *Neoscytalidium dimidiatum, Neoscytalidium hyalinum* (a white variant of *N. dimidiatum*), *Hortea (Phaeoannellomyces) werneckii* and *Piedraia hortae.* Some

non-dermatophyte moulds can infect nails damaged by physical trauma, disease or pre-existing infection with a dermatophyte. There are many non-dermatophyte moulds that have been implicated in nail infection, therefore isolation of a mould from a nail specimen should be reported only if certain strict criteria are met because contamination of nail samples with mould spores is common. A non-dermatophyte mould accounts for the diagnosis in less than 5% of infected nails. *Candida* species, particularly *C. parapsilosis, C. guilliermondii* and *C. albicans* have been reported as a significant cause of nail infections especially in finger nails where people's hands are immersed in water regularly. Tiny flakes of skin from the chest or back are suggestive of pityriasis versicolor in association with hypo or hyper-pigmentation.

The skin may be a target organ for the development of metastatic, presumably haematogenous, infection with a variety of fungi causing systemic mycoses in those hosts that are immunocompromised (filamentous fungi such as *Aspergillus* and *Fusarium* species, *Candida* species, *Cryptococcus* species etc).

Sometimes, fungi such as *Sporothrix schenckii* or *Cryptococcus neoformans* may gain access to the tissues via percutaneous inoculation, and may then cause locally invasive or possibly systemic disease. Cryptococcosis in patients with renal transplants and HIV infection may present with cutaneous lesions.

Wounds may also be contaminated by moulds such as *Aspergillus* and *Alternaria* species, and mucoraceous moulds. In most cases the growth of the fungus will only be locally invasive but may cause extensive tissue necrosis.

Occasionally, patients with primary (invasive, systemic) mycoses are encountered whose presentation is with infection of the skin or mucous membranes. Conditions such as histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, or infections caused by *Cladophialophora bantiana* (formerly *Xylohypha bantiana* or *Cladophialophora bantianum)* or *Talaromyces* (formerly *Penicillium*) *marneffei* may also present with cutaneous manifestations of disease.

If the presence of the agents of these diseases is known or might reasonably be suspected, then clinical material and cultures must be handled under Containment Level 3 precautions.

Clinical manifestations of superficial fungal infections[5,6]

Tinea barbae[7]

Infection of the beard can be mild or present as a severe pustular folliculitis which can be misidentified as a *Staphylococcus aureus* infection. *Tinea barbae* is often associated with zoophilic dermatophytes such as *Trichophyton verrucosum*, *Trichophyton mentagrophytes* and rarely *Trichophyton erinacei:* the anthropophilic *Trichophyton rubrum* is also encountered[8].

Tinea capitis[9]

Infection of the scalp is usually caused by *Microsporum* or *Trichophyton* species the main causal species is determined by the animal contacts or contacts associated with travel history or local infection prevalence. Infection can range from mild scaling lesions to a highly inflammatory reaction with folliculitis, scarring and alopecia when the lesion is referred to as a kerion. The skin surface and hairs may be involved. The arrangement of the fungal spores in the hair shaft can be diagnostic of the infecting species. The terms used are:

Ectothrix – sheath of arthroconidia (spores) formed on the outside of the hair shaft.

Endothrix – arthroconidia contained within the hair shaft.

Ectoendothrix – spores form around and within the hair shaft.

Favus – hyphae and air spaces form within the hair shaft.

Tinea corporis[10]

This infection is known as "ringworm" of the body and may involve the trunk, shoulders and limbs. Infection may range from mild to severe, commonly presenting as annular scaly lesions with sharply defined, raised, erythematous vesicular edges.

Tinea cruris

Infections of groin, perianal and perineal sites are the most common in adult males. *T. rubrum* and *Epidermophyton floccosum* are the most commonly implicated fungi. Lesions are erythematous and covered with thin, dry scales. Lesions can extend down the sides of the inner thigh and have a raised, defined border, which may have small vesicles.

Tinea favosa (Favus)

This is a severe and chronic condition which is found in Africa and Asia. Typically crusts (scutulae) form around the follicles of the infected hairs which consist of epithelial debris and mycelium. The condition is usually caused by *Trichophyton schoenleinii.*

Tinea imbricata[11]

This is a chronic infection, which is a manifestation of *tinea corporis* and mainly found in the Pacific Islands. It has a very distinctive appearance of concentric rings of overlapping scales. The only causative agent is *Trichophyton concentricum.*

Tinea manuum

Palms and interdigital areas of hands are affected. This condition usually presents as a diffuse hyperkeratosis and is usually caused by *T. rubrum* and other *Trichophyton* and *Microsporum* species. Hands are also a likely site for infection with zoophilic or geophilic dermatophytes particularly if the lesions are inflammatory, and involvement can spread to other body sites by contigious spread and scratching. Other causes of infection resembling tinea may occur at the palms and finger webs due to *Neoscytalidium* species, and finger webs may be susceptible to *Candida* infection.

Tinea pedis (athlete's foot)

Toe webs and soles of the feet are most commonly affected; particularly the spaces between the fourth and fifth toes may show maceration, peeling and fissuring of the skin. Another presentation is a chronic, squamous, hyperkeratotic type with fine silvery scales covering the pink areas of the soles, heels and side of feet ("moccasin foot"). The common agents of tinea pedis are *T. rubrum, T. interdigitale* and *E. floccosum.* An acute inflammatory condition with vesicles, pustules and bullae is also caused by *T. mentagrophytes.* The dry hyperkeratotic presentation on soles and toe webs may be caused by *Neoscytalidium* species, and other causes of toe web infection can include *Candida* species.

Tinea unguium / onychomycosis[12]

Traditionally Tinea unguium described the invasion of the nail plate by dermatophyte fungi, and infection by non-dermatophytes was defined as onychomycosis. However, the term onychomycosis is now accepted as the general term for any fungal infection of the nail.

There are four recognised types of onychomycosis:

1. Distal and lateral subungual onychomycosis is the most common form, usually caused by *T. rubrum.* Characterised by invasion of the hyponychium under the nail bed and sides of the nail followed by spread to the nail plate
2. Proximal subungual onychomycosis, also known as proximal white subungual onychomycosis, is relatively uncommon, again usually caused by *T. rubrum.* The organism invades the nail via the cuticle. This presentation of nail infection is most commonly seen in patients with HIV/AIDS
3. White superficial onychomycosis, relatively rare and caused by fungi invading the upper layers of the nail plate. It presents with well-delineated white "islands" on the nail plate. Most commonly caused by *T. interdigitale*

Often fingernail involvement is associated with HIV infection *Candida* species infections, most commonly caused by *C. albicans.* Candida can infect nails and cause paronychia. Infection of the distal nail plate is associated with Raynaud's disease.

4. Total dystrophic onychomycosis is the end stage nail disease. It may be end result of any of the four preceding conditions

See Table 1 for the list of dermatophytes, moulds and yeasts that cause nail infection.

Pityriasis versicolor (tinea versicolor)[13-15]

This is an infection of the stratum corneum by lipophilic yeasts of the *Malassezia* genus. There is little tissue involvement, and the disease is mainly cosmetic and involves changes in pigmentation of the skin. The organisms of the *Malassezia* complex will not grow on routine mycological media and diagnosis is generally made on clinical appearance as well as the microscopic detection of the yeast cells together with short, curved, non-branching mycelial elements in skin scrapings.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance

therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[16,17]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Medium

Sabouraud medium (glucose peptone medium) is the best for routine fungal isolation. There are many different commercial preparations of this, each of which will have an effect on the final appearance of the dermatophyte colonies, so it is important that laboratories become familiar with the appearance of the different species on their own agar. Plates should be quite thickly poured to prevent drying out during the extended incubation periods. The presence of chloramphenicol is essential to help reduce bacterial overgrowth. Cycloheximide prevents overgrowth of non-dermatophyte moulds, but a medium containing this agent should not be used when infection with a non-dermatophyte mould is likely or suspected. Many laboratories will typically inoculate two Sabouraud plates, one with and one without chloramphenicol.

Incubation

Dermatophytes do not grow well at temperatures above 30°C so it is important that incubators are kept at 28°C. The tolerance range should be set at 26°C - 30°C.

Specimen transport

There are several proprietary brands of transport package available for the collection and transport of skin, nail and hair samples.

Microscopy

KOH preparations are not permanent and the reagent eventually destroys the fungi. The addition of a small amount of glycerol to the preparation will preserve it for several days.

KOH combined with calcofluor white is a more sensitive method, but a fluorescent microscope with appropriate filter is required.

Table 1. Specimen types in which dermatophytes, other moulds and yeasts may be present

Specimen types/clinical manifestation	Pathogenic fungi commonly known to be associated with infection. This list is not exhaustive, and other fungal species may cause infection
Skin:	
Tinea barbae	*T. mentagrophytes, T. erinacei, T. verrucosum, T. rubrum*
Tinea capitis	*M. audouinii*, *M. canis*, *T. mentagrophytes*, *T. rubrum*, *T. tonsurans, T. soudanense, T. violaceum*
Tinea corporis	May be caused by any dermatophyte
Tinea cruris	*T. rubrum, E. floccosum*
Tinea imbricata	*T. concentricum*
Tinea manuum	*T. rubrum, T. mentagrophytes, T. erinacei, M. canis, M. persicolor*
Tinea pedis	*T. rubrum, T. interdigitale, E. floccosum*
Nail:	
Tinea unguium/ onychomycosis	*T. rubrum, T. interdigitale, T. mentagrophytes, E. floccosum* (agents of tinea capitis may also be encountered in the fingernails of individuals with scalp infection) *Acremonium* species, *Alternaria* species, *Aspergillus* species, *Fusarium* species, *N. dimidiatum, N. hyalinum, Scopulariopsis brevicaulis, Onychocola canadensis, Candida albicans, C. guilliermondii, C. parapsilosis, C. tropicalis*
Hair:	
Tinea favosa	*Trichophyton schoenleinii*
Tinea capitis	*M. canis, M. audoinii, T. tonsurans, T. soudanense, T. verrucosum, T. violaceum*

1 Safety considerations[16-32]

1.1 Specimen collection, transport and storage[16-21]

Use aseptic technique.

Collect and send specimens in appropriate CE marked leak-proof containers.

Care should be taken if using a sharp scalpel blade or scissors to collect samples.

Specimens should be collected into folded paper squares secured and placed in a plastic bag or in commercially available packets designed specifically for the collection and transport of skin, nail and hair samples.

Transport specimens in CE marked container in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[16-32]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[24].

If infection with a Hazard Group 3 organism, for example *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Cladophialophora bantiana* (formerly *Xylohypha bantiana* or *Cladophialophora bantianum*) or *Talaromyces marneffei* or an agent of exotic imported mycosis, is suspected, all work must be undertaken in a microbiological safety cabinet under full Containment Level 3 conditions.

Many fungi are known to have allergenic effects so care should be taken to limit dissemination of fungal spores.

10%–30% KOH used in the microscopic examination of dermatological specimens is corrosive. KOH is used to slowly dissolve the sample leaving the fungus cells exposed.

Note: Varying strengths of KOH of 10%-30% are quoted in literature. If 10% or 15% is used samples will take longer to digest and 30% is extremely corrosive. Laboratories should continue to use the strength which they find appropriate.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Skin, hair, nails

2.2 Optimal time and method of collection[33]

Skin

Patients' skin and nails can be swabbed with 70% alcohol prior to collection of the specimen, this is especially important if creams, lotions or powders have been applied. The edges of skin lesions yield the greatest quantities of viable fungus. Lesions should be scraped with a blunt scalpel blade. If insufficient material can be obtained by scraping and being placed in a container, then a swab or sticky tape can be pressed on the lesion and transferred to a clean glass slide for transport to the laboratory ('stripping'). Samples in containers achieve the optimum results.

Nail

Good nail samples are difficult to obtain. It should be specified whether the sample is from the fingernails or toenails. Material should be taken from any discoloured, dystrophic or brittle parts of the nail. The affected nail should be cut as far back as possible through the entire thickness and should include any crumbly material. Nail drills, scalpels and nail elevators may be helpful but must be sterilized between patients. When there is superficial involvement (as in white superficial onychomycosis) nail scrapings may be taken with a curette. If associated skin lesions are present samples from these are likely to be infected with the same organism and are more likely to give a positive culture. Sample from associated sites should be sent in separate packets.

Hair

Samples from the scalp should include skin scales and hair stumps. Cut hairs are not suitable for direct examination as the infected area is usually close to the scalp surface. Scraping for direct examination is the preferable sample collection method, however plastic hairbrushes, scalp massage pads, swabs or plastic toothbrushes may be used to sample scalps for culture where there is little obvious scaling. If sufficiently long, hairs should be plucked with forceps and wrapped in black paper or commercial transport packs together with flakes of skin. Collect specimens other than swabs into appropriate CE marked leak-proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens[33]

Numbers and frequency of specimen collection are dependent on clinical condition of patient. The minimum amount that is acceptable should be enough to cover a five pence piece.

3 Specimen transport and storage[16,17]

3.1 Optimal transport and storage conditions

Collect specimens before antifungal therapy where possible[33].

Specimens should be transported and processed as soon as possible[33].

Specimens should be kept at room temperature and transported and processed as soon as possible although, provided the samples are kept dry, the fungus will remain viable for several months.

Samples should be allowed to dry out and kept at room temperature.

4 Specimen processing/procedure[16,17]

4.1 Test selection

Select a representative portion of specimen for microscopic examination and culture.

4.2 Appearance

N/A

4.3 Sample preparation

Skin, nail, hair

4.4 Microscopy

4.4.1 Standard

Skin specimens

Cut into small (1-2 mm) fragments. Place 5 or 6 fragments in a drop of 10%-30% potassium hydroxide (KOH) on a microscope slide. Cover with a coverslip and leave for 15–20 minutes at room temperature.

If there is insufficient material for both microscopic examination and culture, perform a microscopic examination rather than culture (unless the clinician has already done microscopy). Make a note on the request form that there was insufficient material for culture.

Once skin material has digested, press down the coverslip to squash out the fragments and render them transparent, and blot off excess KOH.

Scan each slide with the x 10 objective. If fungal hyphae are seen, confirm their presence with the x 40 objective.

Dermatophyte infections show septate, sparsely branching hyphae of even diameter, which may develop chains of rectangular spores (termed arthrospores: arising from fragmentation of hyphae). It is useful to note the presence of arthrospores as an indication of the presence of a dermatophyte infection. It is important to remember that up to 35% of dermatophyte-infected nails fail to yield the organism on culture, so careful microscopy is of paramount importance in making the diagnosis.

In cases of pityriasis versicolor the fungus appears as clusters of spherical or sub-spherical cells together with short, unbranched hyphae[13]. This should be reported as "Microscopy suggestive of pityriasis versicolor".

Candida in skin and nail samples will usually appear as oval, thin-walled budding yeasts, budding on a narrow base, together with filaments which may be true or pseudohyphae. Sometimes yeast cells alone are seen.

Nail specimens

Cut into small (1-2mm) fragments or scrape material from both upper and lower surfaces of the nail(s). Place 5 or 6 representative fragments in a drop of 10%-30% KOH on a microscope slide. The specimen should be squashed to obtain a single layer of cells. Cover with a coverslip and put aside to digest for at least 30 minutes at room temperature. Press down the coverslip to squash out the fragments and render

them transparent, and blot off excess KOH. If the specimen consists of more than one piece of material, use some of each for microscopic examination and culture.

Scan each slide with the x 10 objective. If fungal hyphae are seen, confirm their presence with the x 40 objective. Chains of rectangular spores (termed arthrospores: arising from fragmentation of hyphae) are typical of dermatophyte infection. Chains of arthrospores are not usually seen in other mould infections of nails, this is therefore an important feature to note as it may help in the assessment of significance of a subsequent non-dermatophyte mould isolate. It is not unusual to see flattened or distorted hyphae in nail infections, several moulds and dermatophytes may show this morphology.

With the possible exception of *S. brevicaulis*, in which typical flat-based conidia may be formed in air pockets within the nail, other moulds cannot be distinguished from dermatophytes on direct microscopic examination of nail specimens. Non-dermatophyte moulds are sensitive to cycloheximide, so all nail specimens should be cultured on Sabouraud Dextrose Agar with chloramphenicol (SABC) and Sabouraud Dextrose Agar with chloramphenicol and actidione (SABCA) to allow for their growth regardless of microscopy result.

Hair specimens

Cut hairs about 5 mm above the root and place 5 or 6 roots and skin scales in a drop of 10%-30% KOH on a microscope slide. Cover with a coverslip and leave to soften for 20 minutes at room temperature.

Scalp specimens should not be squashed as infected hairs will disintegrate and the diagnostic arrangement of the arthrospores will be lost.

If a hair specimen shows evidence of infection, note the size of the arthrospores and their arrangement as described in table 2 below.

Table 2. Arthrospore size and arrangement

Fungus	Arthrospore size (µm)	Arrangement
M. audouinii	Small 2-5	Ectothrix
M. canis	Small 2-5	Ectothrix
T. mentagrophytes	Small 3-5	Ectothrix
T. erinacei	Small 3-5	Ectothrix
T. verrucosum	Large 5-10	Ectothrix
T. tonsurans	Large 4-8	Endothrix
T. violaceum	Large 4-8	Endothrix
T. soudanense	Large 4-8	Endothrix

Note any microscopic findings on the request form.

Skin strippings[13]

Transparent waterproof adhesive tape is applied to the infected area, peeled off and stuck to a sterile microscope slide for examination. If strippings are received and the clinical diagnosis is 'pityriasis versicolor', the tape should be removed and placed on a

drop of 1% crystal violet on a microscope slide for one minute followed by rinsing in running water. This should be examined microscopically. In cases of pityriasis versicolor, the fungus (*Malassezia* species) appears as short, unbranched hyphae together with the commensal *Malassezia* yeasts.

4.4.2 Supplementary specialised staining technique

If there is ready access to a fluorescence microscope the use of an optical brightener such as calcofluor white or blankophor can enhance the detection of fungal elements in skin, nail and hair specimens.

Skin and hair specimens

Calcofluor white (0.1%) can be used in equal proportion with 10%-30% KOH at room temperature and placed over the specimen on a microscope slide, covered with a coverslip and left to digest for at least 20 minutes. During this time the slides should be protected from light. After digestion the specimen should be squashed to produce a single layer of cells and examined under a fluorescence microscope at emission 360-370 nm for blue-white fluorescence (or at an excitation and emission that is recommended by the manufacturer's instructions). Scrapings from scalps should only be squashed after preliminary microscopic examination has failed to show any spores in hairs.

Nail specimens

It is important that nail samples are pre-softened before the addition of calcofluor white or it will be unable to penetrate the tissue. Place a few fragments of chopped-up nail sample in a small tube, cover with 10%-30% KOH and leave for at least 30min at room temperature to digest. After this time use a pipette to remove the nail sample from the tube, place on the surface of a glass slide, add a drop of calcofluor, cover with a coverslip and press down to produce a thin layer of cells. Examine under a fluorescence microscope at emission 360-370nm for blue–white fluorescence.

4.5 Culture and investigation

4.5.1 Standard

Skin

The skin sample should be cut into 1-2mm fragments and distributed evenly between the two agar plates. One glucose peptone agar plate supplemented with chloramphenicol and cycloheximide and one glucose peptone agar plate supplemented with chloramphenicol only. If there is insufficient material for both plates, inoculate a plate supplemented with chloramphenicol and cycloheximide.

If the clinician mentions the possibility of infection with *N. dimidiatum* then the sample should be plated on cycloheximide-free medium to allow growth of this organism. This agent together with the white variant *N. hyalinum*, is the only non-dermatophyte mould capable of causing dermatophyte-like lesions of the palms, soles and toe-webs.

Tinea nigra, which is caused by the mould *Hortaea werneckii*, is a rare condition which causes dark pigmented areas usually on the skin of the palm and is clinically distinctive from dermatophyte lesions. On microscopy, brown darkly septate hyphae are seen. As this is a non-dermatophyte mould, cultures from patients with suspected *tinea nigra* infection should be processed on cycloheximide-free medium.

Incubate plates at 26°C-30°C for 7-14 days examining weekly. If there is growth of a dermatophyte it should be identified and reported as soon as possible. Plates should then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance before discarding. If *T. verrucosum* is suspected the incubation time may need to be extended as they can take longer to grow. If a culture is growing at 14 days but cannot be identified it should be reincubated and subcultured on to appropriate media to support sporulation.

Negative cultures with positive microscopy can be reported after 7 days, but plates should be re-incubated at 26°C-30°C for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

If there is no growth from material in which fungus was seen on the microscopic examination, the inoculum points should be examined with a plate microscope to ensure that a slow growing *T. verrucosum* is not present (pinprick colonies). If careful examination reveals no growth, send out a preliminary report. Then set up further cultures on glucose peptone agar supplemented with chloramphenicol and glucose peptone agar supplemented with chloramphenicol and cycloheximide and re-incubate the original plate. If there is only sufficient material for one plate the former should be used. If there is no material remaining, a suitably worded final report should be issued.

Nail

The nail sample should be cut into 1-2mm fragments and embedded and distributed evenly between the two agar plates to allow proper colony formation. One glucose peptone agar plate supplemented with chloramphenicol and cycloheximide and one glucose peptone agar plate supplemented with chloramphenicol only. If there is insufficient material for both plates, inoculate a plate supplemented with chloramphenicol and cycloheximide.

Incubate plates at 26°C-30°C for 7-14 days examining weekly. If there is growth of a dermatophyte it should be identified and reported. Plates should then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance before discarding.

Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

If there is no growth from material in which fungus was seen on microscopic examination, send out a preliminary report and set up further cultures on glucose peptone agar supplemented with chloramphenicol and glucose peptone agar supplemented with chloramphenicol and cycloheximide and re-incubate the original plate. If there is only sufficient material for one plate the former should be used. If there is no material remaining, a suitably worded final report should be issued.

Hair

Place the remaining hair roots and skin scales on the surface of a glucose peptone agar plate supplemented with chloramphenicol and cycloheximide. Incubate plates at 26°C-30°C for 7-14 days examining weekly: if there is growth of a dermatophyte it should be identified and reported. Plates should then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the

visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance before discarding.

Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

If there is no growth from material in which fungus was seen on microscopic examination, skin scale fragments attached to the hair should be examined with a plate microscope to ensure that a slow growing *T. verrucosum* or *T. violaceum* is not present (pinprick colonies). If careful examination reveals no growth, send out a preliminary report and set up further cultures on glucose peptone agar supplemented with chloramphenicol and glucose peptone agar supplemented with chloramphenicol and cycloheximide and re-incubate the original plate. If there is only sufficient material for one plate the latter should be used. If there is no material remaining, a suitably worded final report should be issued.

Skin strippings

If two specimens are received, detach them from the microscope slides and place one on the surface of a glucose peptone agar plate supplemented with chloramphenicol and the other on glucose peptone agar supplemented with chloramphenicol and cycloheximide. If one specimen is received, place this specimen on a glucose peptone agar plate supplemented with chloramphenicol and cycloheximide.

Incubate plates at 26°C-30°C for 7-14 days examining weekly: if there is growth of a dermatophyte it should be identified and reported. Plates should then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance before discarding.

Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

All specimens

If growth is evident after incubation for two weeks, but the fungus cannot be identified, it should be sub-cultured to a fresh glucose peptone agar plate, Borelli's lactritmel agar, and/or Malt agar, and/or dermatophyte test medium (DTM) and all cultures incubated for a further week. A urea slope may be helpful to distinguish between *T. rubrum* and *T. interdigitale,* because isolates of *T. rubrum* (with the exception of the granular form) are urease negative. If the isolate still cannot be identified it should be referred to a Mycology Reference Laboratory.

4.5.2 Supplementary

It is inadvisable to use slant cultures but, if they are preferred to reduce the chances of contamination with environmental moulds, it is important to culture sufficient specimen. At least two slants will be required for each sample to allow culture of 20 representative pieces of tissue. An alternative is to seal plates with a proprietary tape. However, unless there are particular problems with air-borne contamination in the laboratory, neither of these measures should be necessary. Heat sterilisation of plate racks after use will help to reduce contamination.

4.5.3 Culture media, conditions and organisms

In certain cases the use of PCR and MALDI ToF may be preferable[34-36].

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Dermatomycosis, onychomycosis, scalp infection	Skin, nail, hair	SABCA*	26-30	Aerobic	7 and 14 d negative microscopy 7 and 21 d positive microscopy	7-21 day as applicable	Dermatophytes and yeasts
Onychomycosis	Skin, nail, hair	SABC**	26-30	Aerobic	7 and 14 d negative microscopy 7, 14 and 21d positive microscopy	7-21 day as applicable	Dermatophytes, moulds and yeasts
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Optional media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
	Skin, nail, hair	2% Malt	26-30	Aerobic	7 and 14 d	7-14 d	Encourages mould sporulation
	Skin, nail, hair	Borelli's lactritmel	26-30	Aerobic	7 and 14 d	7–21 d	Encourages dermatophyte sporulation
	Skin, nail, hair	Dermatophyte test medium	26-30	Aerobic	4 and 7 d	4–7 d	Helps to distinguish dermatophytes but care should be taken as some non-dermatophytes can also cause a colour change
	Skin, nail, hair	Urea[37]		Aerobic	4 and 7 d	4–7 d	Used to distinguish *T. rubrum* (urease negative) from *T. interdigitale* (urease positive)

Other organisms for consideration – occasionally non-dermatophyte fungi cause superficial mycoses, most commonly in nail samples. These include: *Acremonium* species, *Aspergillus* species, *Candida* species, *Chrysosporium* species, *Fusarium* species, *S. brevicaulis*, and *Neoscytalidium* species.

*Sabouraud dextrose Agar with chloramphenicol and cyclohexamide.

** Sabouraud dextrose Agar with chloramphenicol.

4.6 Identification

Organisms should be identified to species level as this may provide important epidemiological information in tracing the source of acquisition of the infection and help to inform therapeutic choices.

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

N/A

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

Unusual dermatophytes should be referred to a Mycology Reference Laboratory for confirmation. Other unidentifiable isolates with good evidence of infection (ie microscopy positive samples isolated in pure culture from several tissue fragments) should be submitted for identification.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomalies that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Laboratories should issue preliminary reports giving the results of direct microscopic examination of dermatological specimens. All specimens sent for diagnosis of ‘tinea/pityriasis versicolor' should be issued with a report stating ‘microscopy suggestive of pityriasis versicolor;’ with final reports following direct microscopy. These reports should be issued as soon as possible after microscopic examination has been completed.

Note: Diagnosis of pityriasis versicolor is on the very distinctive microscopic appearance alone. The causative yeasts of the *Malassezia genus* will not grow on Sabouraud agar without a lipid supplement[13].

5.1.1 Microscopy reporting time

Written report; 24-48 hours.

5.2 Culture

If nothing is seen on microscopic examination and no growth is evident after incubation for one week at 26°C - 30°C, a final report can be issued but the plate should be re-incubated for a further week.

If growth is evident after incubation for one or two weeks, the dermatophyte should be identified and a final report issued.

Non-dermatophyte moulds other than *N. dimidiatum, N. hyalinum* and *H. werneckii* are not normally pathogens of cutaneous tissue. Occasionally moulds such as *Aspergillus* species*, Fusarium* species, *Scedosporium* species, *Talaromyces marneffei* and other dimorphics may be isolated from cutaneous lesions as a result of disseminated or wound infection. There are also a number of other moulds, notably the mucoraceous moulds, which can cause wound infections.

If there is no growth from material after one or two weeks in which fungus was seen on microscopic examination, send out a preliminary report. If there is enough material remaining set up further cultures on glucose peptone agar supplemented with chloramphenicol and cycloheximide and/or glucose peptone agar supplemented with chloramphenicol alone.

If there is insufficient material remaining for a further attempt at culture, send out a final report of the positive microscopy noting that there was insufficient material for repeat culture.

Isolation of a non-dermatophyte mould from nail tissue

Isolation of a non-dermatophyte mould is not considered significant if direct microscopy was negative, exceptions to this might be *S. brevicaulis*, Neoscytalidium or Onychocola. If direct microscopy was positive and no dermatophyte was isolated, but 4 or more colonies of the same non-dermatophyte mould are recovered in pure culture, it should be identified and the result reported. If this occurs in the absence of a positive direct microscopy, the microscopy should be repeated. If the repeat microscopy is negative a further sample should be requested.

However a repeated attempt at isolation of a dermatophyte should be considered if chains of arthroconidia were observed on direct microscopy, as these are more indicative of a dermatophyte infection.

If a non-dermatophyte mould is isolated from a specimen from which a dermatophyte is recovered, the mould is not significant and should not be reported.

Isolation of yeasts from dermatological specimens

Yeast isolates should not be reported unless yeast has been seen on direct microscopic examination or the history accompanying a nail sample specifically includes candida or chronic paronychia and there is heavy growth in culture.

5.2.1 Culture reporting time

Written report at one, two or three weeks stating, as appropriate, that a further report will be issued.

Telephone clinically urgent results when available.

5.3 Antimicrobial susceptibility testing

N/A

6 Notification to PHE[38,39] or equivalent in the devolved administrations[40-43]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[40,41], Wales[42] and Northern Ireland[43].

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UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 39 Investigation of Dermatological Specimens for Superficial Mycoses

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation 18.08.14 – 22.09.14

Version of document consulted on – B 39df+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	08/09/2014	**Professional Body**	UKCMN
Section	Various		
Comment			
a. Page 7 Introduction – dermatophytes 5th paragraph line 1 "The dermatophyte of fungi are classified - remove the of b. Page 8 Line 1 - *S. dimidiatum* should be *N. dimidiatum* and *Phaeoannellomyces (Exophiala) werneckii* should be *Hortaea (Phaeoannellomyces) werneckii* c. Page 12 Table 1 - line up fungus names with clinical manifestation d. Page 15 Specimen processing/procedure "4.4 microscopy 4.4.1 Suggest that UV microscopy with calcofluor staining is the recommended microscopy method, with standard light microscopy relegated to 'if UV microscope is not available e. Page 16 Nail Specimens Line 5 make the same as for skin - press down the coverslip to squash out the fragments and render them transparent, and blot off excess KOH f. Page 17 4.5.1 Standard – Skin Paragraph 2 line 1 - *Scytalidium* (or *Neoscytalidium*) *dimidiatum* (*Hendersonula toruloidea*), change to *Neoscytalidium dimidiatum*. Line 4 change *Scytalidium hyalinum* to *Neoscytalidium hyalinum* g. Page 20 4.5.3 Should it be MALDI ToF not MALDI Tof h. Page 22 5.2 Culture line 6 - Should be *Neoscytalidium dimidiatum* and *Neoscytalidium hyalinum*			
Financial Barriers			
No.			
Health Benefits			
No.			
Recommended Action	a. **ACCEPT** The SMI has been amended. b. **ACCEPT** The SMI has been amended. c. **ACCEPT** The SMI has been amended. d. **NONE** The SMI makes it clear that UV microscopy is the method of choice where it is available.		

	e. **ACCEPT** The SMI has been amended. f. **ACCEPT** The SMI has been amended. g. **ACCEPT** The SMI has been amended. h. **ACCEPT** The SMI has been amended.

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 2			
Date Received	21/08/2014	**Lab Name**	Public Health Wales
Date Received	19/09/2014	**Lab Name**	Truro Microbiology

UK Standards for Microbiology Investigations

Investigation of specimens for *Mycobacterium* species

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Issued by the Standards Unit, National Infection Service, PHE

Bacteriology | B 40 | Issue no: 7.3 | Issue date: 5.10.2020 | Page: 1 of 56

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 7

Scope of document 10

Introduction 10

Technical information/limitations 20

1 Safety considerations 23

2 Specimen collection 24

3 Specimen transport and storage 27

4 Specimen processing/procedure 28

5 Reporting procedure 43

6 Notification to PHE, or equivalent in the devolved administrations 44

Appendix: Investigation of specimens for *Mycobacterium* species 46

References 47

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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	9/5.10.2020
Issue number discarded	7.2
Insert issue number	7.3
Anticipated next review date*	10.01.22
Section(s) involved	**Amendment**
Technical information: Concentration/centrifugation	Section updated to clarify the optional but recommended use of refrigerated centrifuges. The following sentence from the WHO guidance on recovery of mycobacteria has been added: "The rate at which mycobacteria sediment is critically dependent on time of centrifugation and relative centrifugal force applied to the specimen. A longer centrifugation time can offset a lower relative centrifugal force, but increased centrifugation time increases the temperature of the specimen, which leads to additional killing of mycobacteria (hence, a refrigerated centrifuge is highly recommended)" Reference: "Boujtita N. Concentration of Mycobacteria in Clinical Samples using the Thermo Scientific General Purpose Centrifuge with 8x50 mL Individually Sealed Rotor. 2011", which was used previously have been removed and a new reference has been added to support the rational for the new sentence.

Amendment number/date	8/28.02.18
Issue number discarded	7.1
Insert issue number	7.2
Anticipated next review date*	10.01.20

Section(s) involved	Amendment
Section 4.5.1.	In the section "Decontamination of specimens using 0.5N Sodium Hydroxide (NaOH 4% w/v) / modified Petroff method", the correct final concentration equivalence of 4% NaOH has been updated to 1N.

Amendment number/date	7/22.01.18
Issue number discarded	7
Insert issue number	7.1
Anticipated next review date*	10.01.20
Section(s) involved	**Amendment**
Section 4.5.5.	The "incubation" column for automated liquid systems has been updated with information for users to follow manufacturer's instructions.

Amendment number/date	6/10.01.17
Issue number discarded	6.1
Insert issue number	7
Anticipated next review date*	10.01.20
Section(s) involved	**Amendment**
Whole document.	References updated. Section on Whole genome Sequencing and MALDI-TOF MS added. Flowchart updated to reflect the information on the table in subheading 4.5.5. Section 4.5.1 updated to reflect the decontamination procedures used in microbiology laboratories. Technical limitations section updated. Section 4.9 has been updated to mention the reference laboratories that samples could be referred to.

Safety considerations.	This section has been updated accordingly with information on the minimum requirement for when processing of samples in cabinets.
Reporting procedure.	This section has been updated accordingly.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

Public Health England. (2020). Investigation of specimens for *Mycobacterium* species. UK Standards for Microbiology Investigations. B 40 Issue 7.3. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

A50711295

Scope of document

Type of specimen

Sputum, gastric washing, sterile site body fluids (CSF, pleural fluids, ascites, joint fluid), urine, skin or tissue biopsies, bone, bone marrow, bronchoalveolar washings, blood, post-mortem specimens

This UK SMI describes the detection and isolation of mycobacteria from a variety of clinical samples. The use of automated liquid culture systems, plus solid media, is recommended for greater recovery of mycobacteria. The combined application of both phenotypic and molecular technologies gives the most efficient approach to the laboratory diagnosis of tuberculous and non-tuberculous disease.

Management, prevention and control of tuberculosis (TB) are not covered by this UK SMI, but are described in Tuberculosis, the recently updated National Institute for Health and Clinical Excellence (NICE) guideline, NG33[1]. This UK SMI complements the recommendations made in the guidance for the prevention and treatment of tuberculosis, issued by the Department of Health and the collaborative strategy for England 2015 to 2020, published by Public Health England and NHS England[2,3].

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

The genus *Mycobacterium* is a member of the family Mycobacteriaceae and consists of over 100 species and 10 subspecies of which a few have been reclassified to other species within the family[4].

TB is caused by members of the *Mycobacterium tuberculosis* complex (MTBC); in humans this is predominantly *Mycobacterium tuberculosis,* though less often by other members of the complex such as *Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canettii* or *Mycobacterium caprae.* Strains of the live vaccine *Mycobacterium bovis* BCG, which are also used intra-vesically in the treatment of bladder cancer, can occasionally cause disease in patients who are immunocompromised. The two other species currently in the complex*, Mycobacterium microti* and *Mycobacterium pinnipedii* are almost exclusively associated with different mammalian hosts, but a few cases have been reported in patients who are immunocompromised[5-7]*.* Non-tuberculous mycobacteria (NTM) are also increasingly encountered as a cause of disease in humans. Not all persons infected with tubercle bacilli develop disease, and not all those that are infected become infectious to others. Overt disease may develop months or years after the initial exposure. Patients with evidence of potential acquisition of MTBC, for example, on the basis of a positive tuberculin skin test and/or an interferon gamma blood test, but with no symptoms of active disease nor positive sample for MTBC may have latent TB infection (LTBI)[1]. Disease due to tuberculosis may occur in virtually any organ of the body, but is most common in the lungs and infection is characterised by caseating granuloma formation.

Initial infection in a person by MTBC organisms is termed primary tuberculosis. The lesion in the primary infection arises at the site of entry of the organism, which is usually the lung, although the tonsils, intestines or the skin may be involved. Lymph nodes will also be infected at this stage (primary complex). The tuberculin skin test (TST, Mantoux test) becomes positive at 3-8 weeks after infection, and marks the

development of cellular immunity and tissue hypersensitivity. This test is useful in detecting latent disease. Interferon gamma release assays are also available. These tests have sensitivity at least equal to TSTs with superior specificity[8]. Foci developing in the endothelium of blood vessels may rupture leading to disseminated or miliary tuberculosis. Post-primary tuberculosis develops either as a result of reactivation of organisms in a 'healed' primary lesion or because of exogenous re-infection. Post-primary tuberculosis usually occurs five or more years after the primary infection and may affect children as well as adults. Infection with *M. tuberculosis* only progresses to clinical disease in a minority of cases. Patients who are infected with HIV are predisposed to reactivation of latent TB infection, and also to a rapid progression of recently acquired infection. Other predisposing factors may be vitamin D deficiency, which is frequently seen in immigrants and patients becoming immunocompromised due to other causes[9,10].

Mycobacterial cell walls contain mycolic acids, which are long chain fatty acids. These acids prevent ready access of commonly used dyes, and special procedures (for example, heat or detergent) are required to enable dye to penetrate mycobacterial cells. However, once the cells have taken up the stain, it is not subsequently removed by the usual acid-alcohol solvents – and hence mycobacteria are termed acid-alcohol fast bacilli (AAFBs), or now more commonly (AFBs)[1,2,11,12]. This staining characteristic is very important in the microbiological and histological diagnosis of diseases caused by mycobacteria[1,2,11,12].

In view of the contribution of TB to human disease globally, international standards regarding the diagnosis and management of TB have been produced by a collaborative body, including the World Health Organization (WHO), which have then been adapted for application in the European Union; as well as there being authoritative American guidelines[11-14]. PHE has produced a position statement on the use of molecular testing in the diagnosis of TB in England, Scotland and Wales[15].

Mycobacterium tuberculosis complex

Pulmonary tuberculosis

Initial infection occurs by inhalation of droplet nuclei. Once *in situ*, the organisms may be ingested by host phagocytic cells where they may remain viable and may even continue to replicate. From this primary focus (Ghon focus), the organisms spread via the lymphatics to the lymph nodes and may reach the blood stream, infecting the lung and other organs. In the majority of individuals, the granulomatous foci may heal. However, they may continue to harbour viable organisms. Factors such as immunosuppression, alcoholism, malnutrition and ageing may contribute to a failure to contain the infection.

Diagnosis of pulmonary TB is usually based on a combination of clinical and chest X-ray findings, with or without acid fast bacilli (AFB) being seen on microscopy of sputum (a positive smear), which may justify starting therapy whilst awaiting culture results[1,11,16]. Every effort should be made to obtain adequate specimens for culture as *in vitro* susceptibility testing of *M. tuberculosis* isolates is becoming increasingly important in the context of emerging drug resistance and genotyping may provide evidence of epidemiological links[1,12,17]. Three separate sputum samples for AFB microscopy and culture should normally be collected if available - although on the basis of the comparative increase in diagnostic yield, the WHO has now reduced its global recommendation from three to at least two serial samples for detection by direct

smear so as to reduce time to diagnosis, accelerate initiation of treatment as well as reduce workload where laboratory resources are limited[1,2,6,11,13,16,18,19].

Extra-pulmonary tuberculosis

Lymphadenitis

Lymphadenitis is the most common form of extra-pulmonary mycobacterial infection, and when caused by tubercle bacilli it was previously referred to as scrofula. Cervical lymph nodes are most commonly infected, although any lymph node may be involved[20-22]. Abscesses may develop with sinus formation. Cervicofacial lymphadenitis in young children may often be due to non-tuberculous mycobacteria, usually *Mycobacterium avium*[23,24].

Miliary tuberculosis

Miliary tuberculosis was the term first used to describe the resemblance of infected lesions (on chest X-ray) to millet seeds, but is now generally used to describe all forms of progressive disseminated haematogenous tuberculosis[25]. The number of cases seen is increasing due to the increase in patients who are immunocompromised as a result of HIV.

Neuro-tuberculosis - Tuberculous meningitis

Tuberculous meningitis (TBM) is usually caused by the rupture of a sub-ependymal tubercle to the sub-arachnoid space, rather than by direct haematogenous seeding of the meninges. The clinical findings usually begin with malaise, intermittent headache and low-grade fever; followed, within 2 to 3 weeks, by protracted headache, vomiting, confusion, meningism and focal neurological signs[25]. Mortality is greatest in patients aged <5 years or >50 years, and in patients whose illness has been present for more than two months. Diagnosis of TBM relies heavily on clinical suspicion. Although the typical CSF picture in these cases is a raised white blood cell (WBC) count with a predominance of lymphocytes, white cells may be absent, or in some cases, polymorphonuclear cells may predominate. CSF protein is usually elevated. Diagnostic algorithms have been developed to assist in the early identification of patients at greatest risk of TBM[26]. In such cases, the chances of obtaining a positive smear and culture result are increased when a large volume of CSF (> 6mL), or repeated CSF specimens are submitted for examination[26].

Gastrointestinal tuberculosis

Gastrointestinal tuberculosis was commonly found in the pre-antimicrobial era in patients with advanced pulmonary disease, and resulted from swallowing infectious lung secretions[27]. It can be caused by *M. tuberculosis*, or by *M. bovis* (which may follow ingestion of infected unpasteurised milk[28]). Diagnosis of gastrointestinal TB is often made endoscopically. Biopsy tissue from the organ involved yields the highest numbers of organisms for AFB smear and culture[1].

Peritoneal TB

Peritoneal TB may occur in either the ascitic (exudative) or adhesive (dry) forms. The ascitic form is characterised by the presence of free fluid, the adhesive form resulting in fibrous adhesions and most frequently abdominal swelling[28].

Genitourinary tuberculosis

Genitourinary tuberculosis is rare before puberty, and is more common in males[29]. The interval between infection and development of active renal disease is usually very long (years or even decades). As the infection progresses, kidney lesions may caseate, discharging viable AFBs to the renal pelvis and ureter, and infection may therefore spread to the bladder[30]. The patient may complain of frequent micturition which may be accompanied by dysuria or haematuria, and urinalysis will often show proteinuria, haematuria (in up to 50% cases) and 'sterile' pyuria (in over 80%)[12,31].

Bone and joint, including spinal, tuberculosis

Bone and joint, including spinal, tuberculosis is usually a result of haematogenous spread to the bone from a primary pulmonary infection[32]. Predisposing factors include compromised immunity and intravenous drug use. Spinal TB is the commonest manifestation of bone and joint TB, followed by involvement of large weight bearing joints such as the knee or hip. Diagnosis of bone and joint TB is usually made clinically and radiographically, coupled with a suitable biopsy sample for histology and AFB smear and culture. Spinal TB (Pott's disease) occurs most commonly in the lower thoracic and upper lumbar areas[33]. Occurring mainly in older children, young adults and the elderly it results in vertebral collapse with consequent spinal deformity. Tuberculous psoas abscess arises from disease of the thoracic or lumbar spine, and spreads within the sheath of the psoas muscle, sometimes as far as the thigh.

Bacteraemia

Low level bacteraemia often occurs with mycobacteria, and is part of the natural history in an infected individual contributing to the occurrence of TB in other organs[34]. However, detectable mycobacteraemia is comparatively rare except in patients who are otherwise markedly immunocompromised, such as those with HIV-AIDS[35-38]. In AIDs patients from developed countries, this was most often *Mycobacterium avium* complex; in those from sub-Saharan Africa it could be MTBC[37-39]. The incidence of these mycobacteraemic disseminated infections is now much less when effective anti-retroviral therapy is available[40]. *Mycobacterium* species have also been isolated from the blood of other patients who are immunocompromised such as those with leukaemia, and occasionally from patients who are not overtly immunosuppressed[35].

Multi-drug resistant tuberculosis (MDR-TB)

In recent years, cases of single drug and multi-drug resistance in TB have been increasingly reported throughout the world[41,42]. *M. tuberculosis* becomes drug resistant by spontaneous random mutation[43]. Factors associated with drug resistance include incomplete and inadequate treatment, and failure to adhere to the prescribed treatment schedule. Primary resistance is defined as occurring in patients who are infected with a strain that is already resistant. Secondary resistance occurs when resistant mutants of an initially drug sensitive organism emerge during the course of an infection, usually due to inadequate chemotherapy. Multi-drug resistant (MDR) TB strains are resistant *in vitro* to isoniazid and rifampicin. In addition to conventional phenotypic sensitivity testing methods, a proportion of genetic mutations associated with resistance may be identified by various molecular methods. Such methods may be applied to an isolate or direct to a specimen; thus providing earlier identification of the presence of drug resistance. Treatment regimens for MDR-TB tend to be less effective, and more prolonged, than therapy for drug sensitive TB. Strains of extensively drug resistant tuberculosis (XDR-TB) are also becoming more common

particularly in certain geographical regions[44]. These organisms, in addition to being MDR, are also resistant to any fluoroquinolone and at least one injectable 'second line' agent, namely amikacin, kanamycin or capreomycin[45]. Cases of totally drug resistant tuberculosis have now also been reported[46].

Non-Tuberculous Mycobacteria (NTM)

These *Mycobacterium* species have been variously referred to as 'non-tuberculous mycobacteria,' 'environmental mycobacteria,' 'anonymous mycobacteria,' 'atypical mycobacteria,' and 'opportunistic mycobacteria.' NTM are ubiquitous in nature, have a varied spectrum of pathogenicity for humans, are not transmitted from person to person (except for *Mycobacterium abscessus* in which there is evidence to suggest that cross infection between patients is possible) and are often resistant to classical anti-tuberculous chemotherapy[47-51]. However, correlation of *in vitro* resistance with *in vivo* efficacy remains much less defined for slow growing NTM than for MTBC[50]. Over 100 species of mycobacteria have now been described with many recognised as obligate or opportunistic pathogens of man or animals[50,52]. Unlike *M. tuberculosis*, isolation of an NTM species from specimens such as sputum does not equate to disease – the microbiology results need to be interpreted in conjunction with clinical and radiological findings[50]. The taxonomy of this group is continually changing and expanding with the use of new techniques, such as comparative sequencing of the 16S rDNA[52]. The traditional distinction between 'rapid-growing' and 'slow-growing' species (on the basis of the ability of strains to demonstrate clearly visible colonies on subculture on a solid medium in 7 days or less of incubation) remains of clinical and taxonomic value[50,52].

Slow growing species

Mycobacterium avium – intracellulare group (MAI)

The term *M. avium* complex (MAC) is often used for convenience in clinical mycobacteriology to temporarily label strains phenotypically resembling *M. avium*[50]. Separation of many species formerly assigned to this complex (including *M. intracellulare)* is now readily possible. There are currently three species within the MAC and they are *M. avium, M. intracellulare and M. chimaera*[53,54]. Additionally, there are now three valid named subspecies of *M. avium: M. avium* subsp. *avium; M. avium* subsp. *paratuberculosis; and M. avium* subsp. *silvaticum*[50,52,55]. In patients who are immunocompetent, MAI organisms, usually *M. intracellulare*, may invade the bronchial tree, pre-existing areas of bronchiectasis, or old cavities[50]. In immunosuppressed patients *M. avium* and related organisms can cause disseminated infection[40,50]. Infections with *M. avium* may also cause cervical lymphadenitis in young children[23,24,50]. These organisms are often present in water supplies and may contaminate specimens.

M. chimaera, a slow growing NTM found in the environment has been implicated recently in several cases of endocarditis or deep infection following cardiac surgery involving the use of cardiac bypass equipment[56,57].

Mycobacterium gordonae

This is a common aquatic species which has rarely, and disputably, caused disease in patients who are immunosuppressed[50]. It is a common contaminant of clinical samples.

Mycobacterium kansasii

Pulmonary infection is the most common form of disease caused by *M. kansasii*, usually in patients with pre-existing chronic lung disease or pneumoconiosis, although infections can occur in other parts of the body[50]. It is a photochromogen, that is, light is required for colonies to become pigmented.

Mycobacterium malmoense

M. malmoense usually causes pulmonary and lymph node diseases, but disseminated and other extra pulmonary disease has also been reported[50]. Diagnosis of *M. malmoense* infection is as for other mycobacteria, although incubation times may need to be as long as 12 weeks before colonies become visible on solid media[58].

Mycobacterium marinum

M. marinum is the causative organism of 'fish tank' or 'swimming pool' granuloma, a localised skin lesion following contamination of an open wound or abrasion with water from fish tanks, swimming pools and natural areas of fresh or salt water. This specie has an intermediate growth rate with an optimum growth temperature of 28 to 30°C[50].

Mycobacterium ulcerans

M. ulcerans is a cause of skin lesions in various global areas, including Australia ('Bairnsdale ulcer') and South-East Asia; Uganda and other parts of Africa ('Buruli ulcer'); and in Central/South America[50,59]. Infection may lead to a chronic progressive painless ulcer, which can occasionally present in travellers from endemic areas[50,59]. The organism can be difficult to isolate in the laboratory – it is more sensitive to standard decontamination methods than other mycobacteria, it is slow growing (6 to 12 weeks) and requires incubation at 30 to 33°C[50,59]. Molecular methods can be of value in confirming the clinical diagnosis.

Mycobacterium xenopi

M. xenopi is another comparatively common cause of NTM pulmonary disease in certain geographic areas[50,60]. It is thermophilic, with an optimum growth temperature of 45°C; and, similar to *M. malmoense*, grows comparatively slowly at 37°C. It can be isolated from various environmental sources, including hot-water taps, and hence may also be a cause of specimen contamination[50].

Conspicuously fastidious species

Certain other mycobacterial species require specific additional supplements or conditions to be cultured successfully in the laboratory. These include *Mycobacterium genavense* (for example, mycobactin J) and *Mycobacterium haemophilum* (haemin or other iron containing compounds), and both species have shown to be associated predominantly with patients who are immunocompromised, including those who are infected with HIV[50]. However, *M. haemophilum*, which has an optimum growth temperature of 28 to 30°C, can also cause lymphadenitis in otherwise healthy paediatric patients[24,50]. *Mycobacterium leprae*, the causative agent of leprosy, cannot currently be cultured *in vitro*.

Rapid growing species[50,52,61]

Mycobacterium abscessus, Mycobacterium chelonae, Mycobacterium fortuitum

These, and related species, are well recognised as the cause of skin and soft tissue infections[50,61,62]. These mycobacteria may infect long-term vascular catheters and

other medical devices[50]. Such organisms have been found in lavage fluids obtained by bronchoscopy and may be associated with false positive diagnoses. Although variation is found in some subspecies, the optimum growth temperature of these organisms lies between 30 to 33°C.

M. abscessus more so than the other non-tuberculous mycobacteria are an increasing problem for the cystic fibrosis patient group[63]. Testing should be considered in cystic fibrosis patients who show deteriorating lung function but where no clear pathogen has been identified[64-66]. The Cystic Fibrosis Trust microbiology standards recommend routine screening of NTM at least once a year for all patients able to produce sputum and all *M. abscessus* positive isolates should be referred to the appropriate reference laboratory for strain typing[47].

Other slow growing and rapid growing *Mycobacterium* species isolated from clinical specimens have recently been identified using the molecular approach. For information and for update on the taxonomy of the *Mycobacterium* species, see link: http://www.bacterio.net/mycobacterium.html.

Mycobacterium and HIV/AIDS

Of all the people who are infected with HIV globally, a third are estimated to also be infected with *Mycobacterium tuberculosis*[67]. Poverty, overcrowding and homelessness are socio-economic factors common to co-infection with both TB and HIV[68]. Patients who are infected with HIV are predisposed to reactivation of latent TB infection, and also to a rapid progression of recently acquired infection[69]. There is a need for a coordinated approach in the diagnosis and treatment of patients with co-infections[70].

A variety of non-tuberculous mycobacterial species have been isolated from systemic infections in patients who are HIV positive, the most common being MAI – however, the incidence of such disseminated infections is much reduced with the use of highly active anti-retroviral therapy[40,50,71].

New technologies for the diagnosis and typing of *M. tuberculosis*

Commercial blood based assays for the diagnosis of latent tuberculosis

Detection of latent tuberculosis is essential for contact tracing and outbreak control. Traditionally the tuberculin skin test (TST, that is, the Mantoux or also previously the Heaf test) has been most commonly used for detection of latent *M. tuberculosis* infections. However, this procedure is fraught with problems, including; variations in interpretative criteria; false positive and false negative results; limited shelf life of the purified protein derivative (PPD); subjective reading of the results; and the unwillingness of some contacts to return for test interpretation[72]. Environmental mycobacteria and the *Mycobacterium bovis*-derived Bacille Calmette-Guérin (BCG) vaccine commonly cause false-positive results[1,72] .

Consequently there has been a need to develop a more reliable, sensitive and specific test for the diagnosis of latent tuberculosis. To address this need, whole blood-based assays have been developed which can detect *M. tuberculosis*-activated T cells or estimate γ-interferon production by these cells, termed interferon gamma release assays (IGRAs) or interferon gamma tests (IGTs)[1,73]. Two commercially available tests are the QuantiFERON® - TB Gold In-Tube and the T-SPOT® *TB* assays. These are used to identify people who are at increased risk of developing TB and where

treatment of the latent infection may be beneficial. Examples include healthcare workers, individuals who have had recent contact with a patient with active tuberculosis and those with underlying medical conditions such as HIV, leukaemia or lymphoma[1].

The assays detect the host response to infection using mycobacterial antigens which are present in *M. tuberculosis,* but not BCG; though they are found in some other mycobacteria, including *Mycobacterium szulgai, M. marinum* and *M. kansasii.* Thus they are unaffected by prior BCG vaccination or exposure to most non-tuberculous mycobacteria. However, these assays cannot distinguish latent TB infection from active or previously successfully treated disease. Both tests can provide a result within 24hr, and can detect latent TB infection with a high level of sensitivity and specificity. Neither of these tests should be used in isolation to diagnose or define active TB disease[73,74]. Revised recommendations regarding the roles of these tests in the diagnosis of LTBI has been published by NICE, and also by the Centers for Disease Control and Prevention in the United States in respect to their appropriate uses to detect *M. tuberculosis* infection[1,73].

Nucleic acid amplification tests

Real-time PCR has the potential to significantly change the current paradigm for mycobacteria identification by decreasing turnaround time for identification from weeks to hours while maintaining or improving upon diagnostic sensitivity and specificity.

Nucleic acid amplification tests (NAATs) for the detection of MTBC, when applied directly to a primary specimen, may be useful in certain situations, for example to confirm rapidly a diagnosis of tuberculous meningitis[26,75]. Although, the accuracy of such NAATs has been found to be superior when applied to respiratory specimens in comparison with other sites, at this time, sensitivity (and to a lesser extent specificity) continues to limit the value of these tests. For TB disease, in an appropriate clinical setting, both in pulmonary and extra-pulmonary sites, a positive NAAT result could be used to rule in the diagnosis, but a negative one could not rule it out[1,11,26]. The technology continues to develop, and a large multinational study demonstrated a sensitivity of 98% (for one platform) when testing sputum smear positive, and 73% for smear negative, MTBC culture confirmed patients with one NAAT per patient. In light of this and other studies, the WHO has made recommendations regarding the application of this method – notably for it to be used as the initial diagnostic test for TB on at least one sputum for individuals who are suspected of having HIV associated TB or MDR-TB. The continuing need for conventional AFB microscopy and culture is acknowledged. In the US, Nucleic acid amplification testing for TB is recommended as standard of care by CDC and the Association of Public Health Laboratories[76]. The evidence was recently reviewed by NICE, and updated recommendations have been issued for the United Kingdom[1]. Where pulmonary TB is clinically suspected, a NAAT, on a suitable primary specimen from someone 16 years or older, should be pursued in the setting of HIV; if rapid information about the presence (or absence) of MTBC DNA would alter the patient's care; or a large contact tracing exercise is being considered[1]. A NAAT should be considered routinely in the setting of suspected pulmonary TB in children aged 15 years or less[1]. In the setting of extra-pulmonary disease, a NAAT on certain primary specimen types; notably CSF, pericardial fluid and material from a lymph node; should be considered as an "additional" test - if the subsequent result would alter case management[1].

Genotypic methods are of critical value in enabling the preliminary rapid identification of a mycobacterial isolate to the complex or species level, as well as in elucidating the taxonomy of the genus[2,52]. They can also be used to diagnose infections due to fastidious mycobacterial species which are difficult to culture in the laboratory[50,59].

NAATs are also of value for the rapid detection of *M. tuberculosis* genetic mutations associated with drug resistance (notably rifampicin, but also isoniazid; and potentially the fluoroquinolones or aminoglycosides if querying XDR)[42,77-80]. In the United Kingdom it has been recommended that such a test to detect rifampicin resistance should be performed on a suitable sample from patients in whom not only TB is clinically suspected but also for whom one or more significant risk factors for MDR-TB is identified. The factors specified are if the patient has had prior treatment for TB, notably if known to have been poorly adherent; contact with a known case of MDR TB; or birth or residence in a country for which the WHO reports a high proportion (5% or more) of new TB cases are MDR[1].

A commercially available DNA line probe assay test, Genotype Mycobacterium common mycobacteria (CM), targeting the 23S rDNA region, has been developed. This assay has been reported to be sensitive and specific for the identification of most *Mycobacterium* species. An important advantage is the possibility to detect mixed infections. However, this assay has not been able to separate some members of the NTM group. It is also known to misidentify some strains of *M. abscessus* as *M. chelonae*[81,82].

Adenosine Deaminase (ADA) Assay

Adenosine deaminase (ADA) is a purine-degrading enzyme that catalyses the deamination of adenosine in an irreversible manner, which results in the production of inosine[83].

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH_3$$

Adenosine deaminase (ADA) increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens. ADA can be detected in body fluids such as pleural, pericardial and peritoneal fluids. It has been developed and widely used for the rapid and early diagnosis of TB, especially in the case of negative AFB smears from body specimens[84,85].

Genomic typing analysis of *M. tuberculosis* isolates from TB outbreaks and clusters

Sporadic outbreaks of tuberculosis among human populations are a major threat to public health both in industrialised and developing countries. Early and accurate detection of the outbreak strains is paramount in the management and control of potential TB outbreaks. Various genomic based methods have been described[86]. The chromosome of *M. tuberculosis* contains loci at which there are serially repeated genetic elements, with the number of repeats varying between different strains. These numbers can be determined for each isolate to generate a digital profile which can then be compared. These loci have been termed exact tandem repeats (ETR), variable number tandem repeats (VNTR) or mycobacterial interspersed repetitive units (MIRU), and the method has been called mycobacterial interspersed repetitive unit – variable number tandem repeat (MIRU-VNTR) typing[87,88]. In the United Kingdom, 24 different loci are currently analysed for at least one *M. tuberculosis* isolate from each

new patient. *M. tuberculosis* genotyping has been shown to be of value in various settings, including in the identification (or exclusion) of possible incidents of laboratory cross-contamination; the investigation of perceived outbreaks; as well as in the analysis of patterns of *M. tuberculosis* transmission within and between communities[12,87-91]. Advice has been produced by PHE for the interpretation and investigation of clusters generated by the national strain typing programme in the United Kingdom, as well as there being guidance for the application of genotyping results by the CDC.

MALDI-TOF Identification of *Mycobacterium* species

Matrix-assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectroscopy analyses 16s ribosomal proteins. It compares the mass peaks achieved by the test strains against those of known reference strains to facilitate identification. It is possible for an organism to be identified within 20 min, and is increasingly being used in microbiology laboratories to provide a robust organism identification system. Studies have been carried out on the benefit of this technique in the identification of mycobacterial species and it has been found to be a reliable means of distinguishing between closely related species within the group as well as between members of various mycobacterial complexes from culture of clinical specimens[92-94]. MALDI-TOF provides a rapid means of identification for this important group of pathogens, potentially allowing accurate treatment regimens to be started earlier, however further validation is required to enhance existing databases and standardise the method thereby improving interlaboratory reproducibility; and it is not currently used routinely[95].

Whole Genome Sequencing (WGS)

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs. This has been used successfully to detect microevolution within *M. tuberculosis* strains as well as to delineate outbreaks of tuberculosis with unprecedented resolution[96-100]. WGS also generates extended results regarding MTBC strain susceptibility to certain drugs – which currently could be used as genotypic “predictions” whilst awaiting conventional phenotypic results[100].The use of WGS has also shown the frequent transmission of multidrug resistant NTM between patients with cystic fibrosis despite conventional cross-infection measures[49].

Note: There are specialist laboratories in the UK that offer the WGS service on request. It should also be noted that it is anticipated that this will form part of the routine analytical service on AFB isolates, provided by PHE’s national mycobacterium service. Other arrangements are in place in the other administrations.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[101,102]

UK SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.”

Culture of samples

Decontamination

Specimens submitted for mycobacterial culture fall into two categories: specimens normally contaminated with resident flora, and specimens from normally sterile sites. Contaminated specimens require a decontamination step before culture to reduce the likelihood of overgrowth by organisms other than mycobacteria. Excessive decontamination of specimens should be avoided as, although mycobacteria are more resilient than other bacteria to the decontaminating agents used, they are not entirely so, and hence this can produce false negative results[12]. Five different methods are described for the decontamination of samples, but there is not much evidence that one method is optimum and laboratories should select the method that they prefer – which may vary with patient and specimen types. Other processing techniques have also been described which perform well in comparison to the widely-used NALC-NaOH procedure, described in 4.5.1.1; and may also be suitable if compliant with the liquid culture system in use, after local validation[6,103,104].

Sodium pyruvate

It should be noted that although sodium pyruvate enhances the growth of *M. bovis,* it may inhibit some non-tuberculous *Mycobacterium* species.

Concentration/centrifugation

When centrifuging specimens, appropriate time should be added to the total spinning time to allow the centrifuge to reach the appropriate speed and for subsequent braking time.

Concentration of sputum samples, for example by centrifugation, increases the sensitivity of initial microscopy[105].

It should be noted that when using a centrifuge (for all decontamination methods applied to clinical specimens), it is important to ascertain that the rotor reaches and maintains the required RCF of 3000*g* for 15 minutes in order to obtain good recovery of the mycobacteria. The rate at which mycobacteria sediment is critically dependent on time of centrifugation and relative centrifugal force applied to the specimen. A longer centrifugation time can offset a lower relative centrifugal force, but increased centrifugation time increases the temperature of the specimen, which leads to additional killing of mycobacteria (hence, a refrigerated centrifuge is highly recommended)[106].

Cross contamination

False positive cultures, due to cross contamination in laboratories, have been reported and the median false positive rate has been 3.1 %[90]. Cross contamination should be avoided by the use of individual pipettes, single aliquots of decontaminants and other additives whenever possible. Any additives to the sample including water should be sterile.

It is recommended that reagents (such as mucolyse, NaOH and neutralising buffer) should not be dispensed directly from stock bottles but should be prepared and pre-dispensed into appropriate sterile containers aseptically, ready for use. This should be done in order to avoid problems with cross contamination due to inappropriate use of stock bottles of reagent. Laboratories can reduce potential contamination rates by ensuring that staff comply with the local guidelines set out on the preparation of reagents, handling of specimens, checking pH readings of solutions used in decontamination and sterilisation records of laboratory equipment. Contamination rates should be monitored on an ongoing basis.

Automated liquid culture systems

The automated liquid culture systems available in the UK have been tested for their ability to detect a wide range of both slow and rapidly growing mycobacteria; however, reliance should not be placed on these systems alone for the isolation of all mycobacterial species, particularly when investigating patients who are immunocompromised[50]. Their limitations lie in a single incubation temperature and the difficulty of providing the growth additives necessary for certain very fastidious species. Advice may be sought from the Reference Laboratories or relevant system manufacturer. Rare isolates of *M. tuberculosis* are recovered only on egg-based media, such as a Löwenstein Jensen slope[12].

Incubation temperature

The temperature of heat mats should be monitored because plates that are too hot will result in loss of sensitivity in staining, and hence false negative smears[107,108].

Staining of slides

Laboratories that perform TB smear tests should ensure that the smeared slides are fixed at the correct temperature (65 to 75°C) for a suitable period (until dry, approximately 10 minutes in most circumstances, and not exceeding one hour) as recommended in the TP 39 – Staining procedures. This is because prolonged heat fixation or use of higher temperatures than recommended may decrease the sensitivity of detecting alcohol and acid fast bacilli (AAFBs), with associated risk of false negative smears)[107].

Volume of sample

A minimum volume of 5mL is required for reliable NAAT testing of clinical specimens[2,19]. However submitting more volume of clinical specimens will increase sensitivity.

Accreditation

Laboratories should be registered with UKAS. They must include mycobacteriology investigations within their declared scope[2].

Quality control

The quality of commercially available egg-based solid media and liquid media should be guaranteed by the manufacturer. However, storage conditions especially during transit to the laboratories may not be optimal and so it is advisable to revalidate new batches of media received into the laboratory before routine use.

Egg-based media are robust and retain their sensitivity unless exposed to direct sunlight or prolonged high temperatures. Liquid media are more prone to contamination by mycobacteria other than *Mycobacterium tuberculosis* or by other bacteria.

It should be noted that in-house preparation of liquid media and reagents are very susceptible to contamination and so aseptic techniques and utmost care must be taken when preparing these.

Misidentification using GenoType Mycobacterium (CM/AS) assays

This assay has been reported to be sensitive and specific for the identification of most *Mycobacterium* species. However, this assay has not been able to separate the members of the NTM such as some strains of *M. abscessus* as *M. chelonae*[81,82].

1 Safety considerations[101,102,109-123]

1.1 Specimen collection, transport and storage[101,102,109-112]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[101,102,109-123]

Hazard Group 3 organisms.

All *Mycobacterium tuberculosis* complex organisms are notifiable to PHE under the Health Protection (Notification) Regulations 2010.

Mycobacterium tuberculosis can cause laboratory-acquired infections (LAIs). The most important route for transmitting LAIs is through aerosols. As a minimum it is recommended that the processing of all samples, including respiratory samples that may result in generation of aerosols should be processed in a microbiological safety cabinet in Containment Level 2 conditions with additional precautions to minimise risk of aerosols production in accordance with the relevant risk assessment, ACDP and HSE guidelines[115].

However, if processing diagnostic samples that are assessed to be at higher risk of containing hazard group 3 organisms such as *M. ulcerans*, *M. microti* and *M. tuberculosis,* this must be undertaken under appropriate containment conditions as determined by local risk assessment. The nature of the work that may dictate that full Containment Level 3 conditions should be used are; the preparation of sputum smear slides for microscopy, treatment of specimens (such as sputum) before culture, processing of positive blood cultures (MGIT) and DNA extraction from clinical specimens (for molecular techniques such as DNA identification, detection of gene mutations related to drug resistance or possibly genotyping) in order to comply with COSHH 2004 Schedule 3 (4e). If performing the MALDI-TOF test procedure as an example, ensure that the manufacturer's instructions are adhered to on how to use the different types of matrices for MALDI-TOF MS extraction as some are associated with significant occupational hazards such as eye, skin and respiratory toxicity. For more information, see TP 40: Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS) test procedure.

Use disinfectants according to manufacturer's instructions to disinfect surfaces of microbiological safety cabinets, and for wiping the exterior of items of equipment. Phenolic disinfectants are not supported by the Biocidal Products Directive 2001, and therefore no longer available in the UK[124]. Alternatives are available which have varied efficacy of inactivation against *Mycobacterium* species[125]. Public Health England has made recommendations for possible alternatives to phenolic disinfectants including hypochlorites and chlorine dioxide[126]. There is also evidence to suggest that 0.35% peracetic acid (Nu-cidex A and B) is rapidly mycobactericidal[127]. Contact times and concentration for various disinfectants can vary and should be considered. Disinfectants with rapid contact times are desirable.

The NALC-NaOH reagent contains strong alkali and causes severe burns. Gloves and eye/face protection must be worn. NaOH is irritating to the eyes and skin.

The use of hot plates in microbiological safety cabinet in a Containment Level 3 room may alter the airflow through the cabinet. It is important to verify that the air flow is not affected by the hot plate operation and to ensure that operator protection is not compromised.

In settings where equipment/ kit designed for point of care testing (POCT) is used in the laboratory, a risk assessment should be undertaken as to where it can be placed.

Use sealed buckets for centrifugation. After centrifugation, open the buckets in a microbiological safety cabinet.

Transport the discarded material directly to the autoclave when ready for disposal and autoclave immediately.

Use plastic consumables in preference to glass wherever possible.

Note: Heat fixation of smears does not kill *Mycobacterium* species. Handle the slides with care[128] (see section 4.4).

Place and transport specimen containers in holders designed to minimise breakage and spillage.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

Staff must wear personal protective equipment at all times when processing clinical samples in the Containment Level 3 laboratory as well as maintaining safe working conditions.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Sputum, sterile site body fluids (CSF, pleural fluids etc), urine, skin or tissue biopsies, bronchoalveolar washings, post-mortem specimens, gastric washing, bone marrow, blood, bone

2.2 Optimal time and method of collection[129]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[129].

For the initial diagnosis of mycobacterial infection all specimens should be fresh and taken, whenever possible, before anti-tubercular treatment is started. ‘Other’ antimicrobials may also have significant anti-mycobacterial activity, notably the fluoroquinolones such as ciprofloxacin, levofloxacin or moxifloxacin, and the macrolides such as clarithromycin or azithromycin.

Use appropriate hazard labelling according to local policy.

Refer to the relevant HSE/COSHH guidelines on the collection and safe handling of specimens likely to contain Hazard Group 3 organisms.

Aerosol generating procedures, such as bronchoscopy or sputum induction, should be performed in an appropriately engineered and ventilated area[1].

Specimens other than blood

Specimens other than blood or bone marrow should be refrigerated if transport to the laboratory or specimen processing is delayed for >1hr.

Gastric washings

Gastric washings should be neutralised by adding approximately 100mg of sodium carbonate to approximately 50mL of the specimen if processing is delayed for >4hr[130].

Blood and bone marrow cultures

Blood and bone marrow aspirate cultures should be transported and loaded into the automated culture system as soon as possible.

Note: These samples should not be collected in EDTA tubes as this inhibits the growth of mycobacteria. Lithium Heparin tubes are recommended.

2.2.1 Correct specimen type and method of collection

Sputum specimens

Sputum specimens should be relatively fresh (less than 1 day old) to minimise contamination. Purulent specimens are best. Two to three samples of ≥5mL should be collected approximately 8 to 24 hours apart with at least one from early morning[1,2,11,13,19].

Samples taken early morning (that is, shortly after patient waking) have the greatest yield. When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline ('sputum induction') before expectoration may be helpful.

Note: Decontaminated and neutralised samples are not recommended as they may lose viability during transit to the laboratory.

Bronchoalveolar lavage/bronchial washings

These may be sent if spontaneous or induced sputum is unavailable or if such specimens are AFB smear negative.

Note: Contamination of the bronchoscope with tap water, which may contain environmental *Mycobacterium* species, should be avoided. Minimum sample size is preferably 5mL.

Gastric washings

Gastric washings are usually used for children where there are problems obtaining sputum. Young children will often swallow their respiratory secretions rather than cough them up. Induced sputum is considered preferable to gastric washings, if possible[1]. Collect samples early in the morning (before breakfast) on 3 consecutive days[131]. Preferably, a minimum volume of 5mL should be collected. Aspirates should be promptly delivered and processed to avoid acidic deterioration of organisms (see under neutralisation, section 4.5). Results of direct microscopy on gastric washings can be misleading because other acid-fast bacilli are normally present in the stomach.

Sterile site body fluids

Sterile site body fluids (CSF, pleural fluid, etc) will normally not require decontamination, and can be inoculated directly to neutral media. However, these samples can be assessed for contamination by setting up purity plates. If contaminated, they can be treated with acid and if pure they can be directly

inoculated. Collect aseptically as much (for example >6mL in adults) CSF sample as possible into a CE Marked leak proof container in a sealed plastic bag. If only a small volume is available after initial lumbar puncture, and the findings of cell counts and protein suggest TB meningitis, a second procedure should be considered to obtain a larger volume to improve chances of achieving positive cultures[26].

It should be noted that pleural or pericardial fluids are not very sensitive samples for the detection of *M. tuberculosis,* and that a concurrent pleural or pericardial biopsy taken with the fluid is more useful[12]. A negative result on these fluids does not rule out the diagnosis.

Urine specimens

Urine specimens should be collected in the early morning on three consecutive days in a CE marked leak proof container (that does not contain boric acid), and placed in a sealed plastic bag. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.

Skin, bone, and tissue including post mortem specimens[132]

Specimens of such type should be homogenised, with the exception of bone. It may be necessary to select and cut out a suitable piece of tissue if a large piece is received. Similarly, some pieces of tissue may need to be 'minced' using sterile scissors and forceps before they can be successfully homogenised. Specimens should be collected aseptically and placed in a CE Marked leak proof container without preservatives in a sealed plastic bag, and sterile distilled water added to prevent desiccation. A caseous portion should be selected if possible: the majority of organisms will be found in the periphery of a caseous lesion.

Tissue biopsy specimens received in formalin are unacceptable and should not be processed [133].

Faecal samples

Mycobacterium tuberculosis and *Mycobacterium avium-intracellulare* group have been isolated from faeces, notably in patients who are immunocompromised such as those with HIV-AIDS. However, NTMs can often be isolated from healthy individuals, representing colonisation only[134]. If *M. tuberculosis* is isolated, this may well be due to the ingestion of infected respiratory secretions rather than intestinal disease[134]. The isolation procedure is unreliable and has a low success rate due to the heavy contamination with other bacteria; hence culturing faecal samples for mycobacteria is not recommended in this UK SMI. *M tuberculosis* and NTMs, including MAI, may be isolated from blood cultures in disseminated infection.

Pus or pus swabs

Pus, or pus swabs, should be collected aseptically, and the largest practical sample submitted in CE marked leak-proof container in a sealed plastic bag. Pus is the sample type of choice. Swabs are less preferable as mycobacteria, if present, may adhere to the swab rather than be transferred successfully to the culture media[135].

Bone marrow

As large a sample of bone marrow as possible should be aspirated and added directly to the culture medium in accordance with the manufacturer's instructions.

Blood

For more information on blood cultures, refer to [B 37 – Investigation of blood cultures (for organisms other than *Mycobacterium* species)] but microscopy, sub-culturing and further testing should be handled in accordance with the methods outlined in this UK SMI.

Note: EDTA, even in trace amounts, inhibits the growth of some *Mycobacterium* species and so is not acceptable.

Cultures

Culture specimens could be sent as egg medium slopes or aliquots of liquid medium in appropriate transport containers. At least 5mL is required if grown in liquid medium. For details of how to prepare a culture specimen for referral to the appropriate Reference laboratory, see the link in section 4.9 of this UK SMI.

2.3 Adequate quantity and appropriate number of specimens[129]

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport and storage[101,102]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[129].

Specimens should be transported and received in the laboratory within one working day of collection and processed as soon as possible[2]. Requirements of individual testing laboratories should be referred to.

If processing is delayed, refrigeration is preferable to storage at ambient temperature[129].

3.1.1 Transportation

The terms Category A (for positive cultures) and Category B (for specimens) are limited to classifying samples / microbial cultures being transported to another laboratory (see Table below).

Sample description	Packaging requirement
Category A samples are known or suspected to contain a microbial agent with the following definition “an infectious substance which is transported in a form that if exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease to humans or animals”. The majority are Hazard Group 3 or 4	Assign to UN2814 (Humans) Packaging Instructions P620 Supporting documentation as per ADR Transport as category A ADR licensed courier
For practical reasons to allow referral / reference services to continue a limited number of Category A agents have exempted from being transported as Category A. These are Vero-cytotoxin producing *Escherichia coli* (VTEC), *Mycobacterium tuberculosis* and *Shigella dysenteriae* 1	Assign UN3373 Packaging instruction P650 Send by courier Royal Mail will NOT accept

Category B samples are those that do not meet the definitions of Category A	Assign UN3373 Packaging instruction P650 Post or courier Royal Mail WILL accept

4 Specimen processing/procedure[101,102]

4.1 Test selection

If there is sufficient volume, specimens being processed for AFBs should normally have AFB microscopy (a 'smear') prior to, and in addition to, culture; with the exception of samples received already pre-inoculated into a mycobacterial culture bottle. Direct microscopy of urine is also of questionable value; and moreover, depending on the clinical setting, can be misleading due to the presence of non-tuberculous mycobacteria[12,31].

If sample volume is insufficient for both, culture is usually preferred to microscopy due to greater sensitivity.

It may be appropriate to process a sample for AFBs, depending on specimen type and available clinical details, even if these tests were not specifically requested when submitted[1]. Such specimen types in particular would include pleural biopsies or material from lymph nodes[1].

In addition to conventional AFB investigations, nucleic acid amplification test(s) (NAAT), such as by PCR, may also have been requested - either to detect presence of MTBC DNA and/or genetic mutation associated with drug resistance (see 4.5.4 and the introduction for more details when appropriate to consider[1]). **Note:** NAAT on a primary specimen is an "additional" test to consider - if limited specimen volume, conventional tests should normally take priority.

For certain body fluids, such as CSF or pleural, pericardial or ascetic fluids, where TB is being considered, adenosine deaminase (ADA) assay may also be requested[1].

4.2 Appearance

Sputum specimens

The sputum samples should appear thick and mucoid or clear but with purulent grains. The colour varies from opaque white to green.

Bloody sputum specimens will appear reddish or brown.

Note: clear saliva or nasal discharge is not accepted as a TB specimen.

Sterile site body fluids

Note the presence of any clot and, if present, include it in the processing.

Tissue biopsies

Process the entire tissue if the sample is small.

For larger samples, select caseous portions (if present) as well as the tissue immediately surrounding the caseating areas for smears and culture.

4.3 Sample preparation

For safety considerations refer to Section 1.2.

Please see relevant sections for sample preparation for smear and culture investigation below.

4.4 Microscopy

4.4.1 Standard

Microscopy should be performed, and the result issued within one working day of receipt of the specimen[2].

Microscopy should be performed after homogenisation and before decontamination of samples, or directly from samples.

1. Centrifuge homogenised samples in sealed buckets at 3000 x *g** for 15 min.
2. Carefully discard the supernatant to a discard pot containing an appropriate disinfectant.
3. Prepare a thin smear of the deposit on a single microscope slide and heat-fix on the hotplate (65 to 75°C), for a minimum period of 10min and a maximum of 1hr then place in a rack or other suitable holder[107].
4. Stain using the auramine-phenol method.

*It is important that centrifugation is carried out at an appropriate Relative Centrifugal Force (RCF). This will vary with the centrifuge and is a factor of rotor arm length and rotation speed. This should not be confused with Revolutions per Minute (RPM).

Note: At least a six day service during the normal working days should be provided for smear examination of appropriate samples[2].

4.4.2 Preparation of smears

Sputum smears

Using a plastic loop, spread the treated centrifuged deposit over the slide keeping away from the slide edges[11,12,108]. Avoid making the smear too thick.

Sterile site body fluids

Prepare smears as for sputum above from the spun deposits. In the case of CSF it may be appropriate to build up several layers of material, if sufficient volume.

Tissue

Smears from tissue may be more sensitive when processed by histology, that is, serial sections that are stained by modified Ziehl-Neelsen stain. Direct smears from tissue are possible, but they are usually insensitive. However, where the amount of diagnostic material is limited, culture of fresh tissue is the most sensitive means of making the diagnosis, and provides most information for patient management.

Swabs

Swabs that are received singly should not be examined by direct microscopy as this may lead to contamination. If a pair of swabs is received, or if the tissue is accompanied by a pus swab, then microscopy can be carried out. Swabs are not the

specimen type of choice. If a single swab is received then suspend the contained material in 1mL of sterile distilled water and treat as sputum.

Blood / bone marrow aspirate

Perform microscopy on any broth bottle from an automated culture system which ‘flags’ positive or which is visually positive. With an appropriate safety needle or device, remove a few drops of blood/broth mixture and place on a clean microscope slide. Spread this with a sterile loop to make a thin smear for acid-fast staining.

Urine

Do not pool urine specimens together when prepared for inoculation.

4.4.3 Staining smears

Auramine-phenol staining is more sensitive than that by the Ziehl-Neelsen method, and is therefore more suitable for assessment of smears from clinical specimens[2,11,12,50,105,108,136]. Ziehl-Neelsen staining provides morphological details and is useful for the examination of AFB in positive cultures, and may be used to review results from clinical specimens that are positive with auramine-phenol.

Stain previously fixed positive and negative control slides, with each batch of auramine-phenol and Ziehl-Neelsen stains, before the stains are routinely used.

Note: When staining slides, to reduce the risk of cross-contamination, it is advisable to ensure that they do not touch each other. Both a negative and a positive control slide should be present and tested correctly. Also ensure they are not splashed with tap water prior to staining – this may produce false positive results due to NTMs present in the plumbing. When using stains, the methodology should be supplemented with local COSHH and risk assessments.

For more information on the staining of heat fixed films by auramine-phenol in clinical specimens and staining of heat fixed films by Ziehl-Neelsen stain in positive cultures, refer to TP 39 – Staining procedures.

4.5 Culture and investigation

4.5.1 Treatment of specimens

All methods go through one or more of the stages outlined below:

Pre-treatment (for example centrifugation):

This is not suitable for all specimen types.

Homogenisation:

Improves the sensitivity of culture but is not required for all sample types.

Decontamination and neutralisation:

Removes contamination and balances pH. The timing of the various stages should be reviewed in light of individual laboratory contamination rates. Laboratories using automated culture systems should refer to manufacturer’s recommendations for compatible decontamination methods. Five decontamination/digestion methods which are currently used to process specimens are:

1. Decontamination of specimens using 1N Sodium Hydroxide (NaOH 4% w/v) / modified Petroff method

2. Decontamination of specimens using *N*-acetyl-*L*-cysteine sodium hydroxide (NALC-NaOH)
3. Decontamination of specimens using trisodium phosphate and benzalkonium (TSPB)
4. Decontamination of specimens using H_2SO_4 (0.5N)
5. Decontamination of specimens using oxalic acid (5%)

There is not enough evidence or evaluation data available to recommend an optimum method. The choice of the most suitable method and length of decontamination will vary with the level of contaminants in the specimens. Laboratories should treat samples according to the level of contamination expected in the sample.

There are commercially available specimen digestion/decontamination kits. Follow manufacturer's instructions.

Note: It should be noted that the success of homogenisation and decontamination depends on the appropriate concentration of homogenisation or decontamination solution, the length of exposure time to the organisms, the centrifugation speed and time used to sediment the tubercle bacilli as well as the temperature build-up in the specimen during centrifugation.

Concentration (for example centrifugation):

This is not appropriate for all sample types.

4.5.1.1 Specimens from non-sterile sites

The specimens for non-sterile sites include:

- Sputum
- Gastric washing
- Bronchoalveolar washing/bronchial washings
- Laryngeal swabs (material should be eluted from the swabs using NaOH and the product treated as for sputum. If microscopy is required, two swabs should be sent)

Note: Specimens from otherwise sterile sites such as (CSF, bone marrow, tissue biopsies, pleural, pericardial and peritoneal fluids, surgically resected specimens (excluding autopsy material) pus from cold abscesses, spinal fluid, synovial or other internal body fluids) do not need decontamination prior to culture.

Pre-treatment:

Centrifuge fluid samples, if sufficiently liquid, for example gastric lavage, bronchoalveolar washings. Centrifuge at 3000 x *g* for 15min and discard supernatant into disinfectant, leaving 1mL to re-suspend the pellet.

Homogenisation

Homogenisation can be achieved by one of the following methods:

1. Repeatedly vortexing during the decontamination process until the specimen is fully homogenised
2. Treatment with dithiothreitol (DTT): Liquefy samples with an excess volume of dithiothreitol, shaking or vortexing intermittently until the specimen is

homogenised. Centrifuge at 3000 x *g* for 15min and discard supernatant into disinfectant, leaving 1mL to re-suspend the pellet

Note: The use of DTT allows dilution and facilitates retrieval of micro-organisms present in the sample[137].

3. Treatment with N-acetyl-L-cysteine (NALC): Include NALC at decontamination stage (see decontamination of specimens using NALC-NaOH)

Decontamination of specimens using 4% NaOH (modified Petroff method)[138-140]

NaOH is a commonly used decontaminant and serves as a mucolytic agent but strict adherence to the reduced indicated timing for the modified Petroff method is required as it is slightly more harmful to the tubercle bacilli than to contaminating organisms. However, if using the original Petroff method, timing is extended to 30 minutes and the specimen is neutralised by HCL using a drop of phenol red as indicator before inoculation onto appropriate media.

1. Add the specimen (3-5mL) into the centrifuge tube and an equal volume of 4% NaOH with 0.04g/l phenol red indicator already in it
2. Allow the NaOH to act for 15min at room temperature (20 to 25°C), vortexing at regular intervals (for example every five minutes)
3. Neutralise the specimen with sterile 0.067 M phosphate buffer (pH 6.8) or 20mL sterile distilled water
4. Centrifuge at 3000 x g for 15min

Note: if using 2% NaOH as a decontaminating agent, this concentration is toxic to both contaminants and to some mycobacteria.

Decontamination of specimens using NALC-NaOH[138]:

This method is a “preferred method” for the digestion step because it is the least toxic to mycobacteria and therefore provides the highest yield of positives. It is the most commonly used method in clinical laboratories.

1. Add an equal volume of working NALC-NaOH solution to the specimen in the centrifuge tube. Tighten the screw-cap

   Note: Mix equal volumes of 0.5 N NaOH* solution and $Na_3C_6H_5O_7.2H_2O$** solution (trisodium citrate. $2H_2O$). These can be premade before the day of use. Add 2% NALC on the day of use to the mixed solution when it will be used, as its activity wanes if pre-made and stored.

   *Add 4g of NaOH to 100ml of distilled water to make the NaOH working solution.

   ** Add 2.9g $Na_3C_6H_5O_7.2H_2O$ (or 2.6g if using anhydrous sodium citrate) to 100ml of distilled water to make the $Na_3C_6H_5O_7.2H_2O$ working solution.

2. Agitate the tube on a vortex mixer for not more than 20sec. Invert the tube so that the NALC-NaOH comes in contact with the entire inner surface of the tube. Avoid excessive agitation
3. Allow the tube to stand for 15min at room temperature (20 to 25°C) to decontaminate the specimen
4. Dilute the mixture with sterile 0.067 M phosphate buffer (pH 6.8) containing phenol red indicator and invert several times to mix the contents

5. Centrifuge at 3000 x g for 15min

   Note: If processing sputum, three washes should be done instead of one.

Decontamination of specimens using trisodium phosphate and benzalkonium (TSPB)[139,141]

This method has been found to be a useful procedure as these chemicals are fairly non-toxic to mycobacteria and provide reasonably better mucolysis.

The timing of this process is not critical for viability of tubercle bacilli. Its limitations are its laboriousness and the unavailability of the materials needed as benzalkonium is not easily available.

- Add the specimen into the centrifuge tube and add an equal volume of the TSPB solution
- Tighten the screw-cap and vortex. Allow to stand at room temperature for 30min
- Neutralize the mixture with sterile 0.067 M phosphate buffer (pH 6.8) and vortex briefly to mix the contents

Concentration

Centrifuge at 3000 x *g* for 15min using sealed buckets within the centrifuge. Discard supernatant into disinfectant, leaving 1mL to re-suspend the pellet or re-suspend in 1-2mL sterile 0.067 M phosphate buffer (pH 6.8). (The latter has the added effect of increasing the neutralisation activity).

4.5.1.2 Specimens that are heavily contaminated with Gram negative bacteria

The specimens that are heavily contaminated with Gram negative bacteria include:

- Urine
- Skin or tissue biopsies from non-sterile sites
- Post mortem specimens
- Pus, aspirates and fluids

Pre-treatment

- Centrifuge fluid specimens, for example urine or pus if suitably liquid and sufficient volume in sealed buckets at 3000 x *g* for 15min. Do not pool urine samples
- Open centrifuge buckets and carefully decant supernatant into a discard pot containing a suitable disinfectant
- Cut tissue into small pieces with a sterile scalpel and homogenise down all tissue specimens in a sterile porcelain mortar or tissue grinder. Transfer to a sterile universal. Place a portion of the sample in a sterile bijou and store at ≤ -20°C to allow culture to be repeated if contaminated. Plate out directly

Decontamination of specimens using (0.5N) H_2SO_4[139,142]

This method is used for specimens that consistently produce contaminated cultures when processed with one of the alkaline digestants. A major limitation of this method is that it may inhibit most of the tubercle bacilli in the specimen.

1. Add an equal volume of H_2SO_4 (0.5N) to all fluid/tissue specimens and allow to act for 20 to 30min. Timing of the various stages should be reviewed in light of individual laboratory contamination rates for different specimen types
2. Top up the container with sterile 0.067 M phosphate buffer (pH 6.8) after treatment and centrifuge at 3000 x *g* for 15min
3. Discard the supernatant into discard pot leaving the deposit in approximately 1mL of liquid or re-suspend in 1 to 2mL sterile 0.067 M phosphate buffer pH 6.8 (The latter has the added effect of increasing the neutralisation activity)
4. Neutralise the sediment with 4% NaoH containing a phenol red indicator (the phenol indicator is added so that neutralisation can be verified visually. However, the use of lower working concentrations of NaOH (2%) together with a dilution step should obviate the need for this step)
5. Inoculate the sediment directly onto culture medium (in urine specimens)

Note: It should be noted that the use of phenol red indicator is not compulsory. Users omit the use of phenol red indicator where they have validated their methods. However, some users use this to ensure that neutralisation has taken place especially when training is given to staff.

4.5.1.3 Specimens contaminated with *Pseudomonas* species

The specimens include sputum or other respiratory samples from patients with cystic fibrosis or bronchiectasis that are likely to be consistently colonised with *Pseudomonas* species.

Homogenisation

See Section 4.5.1.1 Specimens from non-sterile sites.

Decontamination of specimens using 5% oxalic acid[139]

This method is used for specimens consistently contaminated with *Pseudomonas* species.

1. Add sufficient amount of 5% oxalic acid to the specimen in a plastic universal container to almost fill the container and vortex
2. Allow the acid to act for 30min (or longer if necessary) shaking intermittently to aid homogenisation and decontamination
3. Centrifuge the specimen at 3000 x *g* for 15min
4. Decant the supernatant into a discard can containing appropriate concentration of disinfectant
5. Neutralise the specimen with NaOH (0.5N) or re-suspend in 1-2mL sterile 0.067 M phosphate buffer (pH 6.8).

Or

Note: If recovering *M. tuberculosis* from swab samples,

1. Cover the swab with 5% oxalic acid for 15min and then transfer into sterile saline for a few minutes
2. This can be later removed and allowed to drain.
3. Neutralise the specimen (swab) with 2 to 3mL of 5% sterile sodium citrate for 5min before inoculation in media

4.5.1.4 Specimens from sterile sites

Cerebrospinal fluid (CSF), blood and other samples from normally sterile sites, or specimens already shown to be free of viable bacteria by culture, do not require decontamination (see below if heavily blood-stained fluid sample, such as from pleura). In addition to culture for mycobacteria, these samples should also be cultured for other pathogens (see B 27 - Investigation of cerebrospinal fluid, B 37 - Investigation of blood cultures for organisms other than *Mycobacterium* species or other UK SMIs as appropriate) to eliminate other causes of infection. This also indicates the absence of other bacteria or reveals the need for decontamination procedures. At this stage consider the need for molecular tests prior to processing.

Centrifuge CSF and other suitable fluid specimens from otherwise sterile sites prior to culturing and smear preparation (3000 x *g* for 15min, as described above). 'Decontamination' (as in 4.5.1.2) of heavily blood-stained non-CSF fluids may also be appropriate prior to incubation in an automated liquid culture system, to reduce false positive signalling of the fluorescence detection system. Use the remainder of both the deposit and the supernatant (if available) for culture. For CSF specimens, after inoculation of appropriate culture media add a liquid culture medium (for example Kirchner's) if available to the original container and incubate with the other inoculated media. Alternatively, tuberculosis growth media from an automated liquid culture system may be used to flush the container.

Homogenise tissues and biopsies and decontaminate if required. Using aseptic procedures, inoculate tissue biopsies and bone that have been cut into small pieces directly to the surface of solid media and to enrichment media.

4.5.2 Automated monitoring systems

Automated culture systems are recommended for faster and easier detection of growth of mycobacteria[2]. Automated culture systems indicate mycobacterial growth by detecting oxygen consumption or CO_2 production. These systems reduce the mean time for detection of growth of mycobacteria, notably in comparison with solid slopes[143-145]. Solid media should be used in addition[2,12,50,146]. Rare isolates of *M. tuberculosis* are recovered only on egg-based media, such as a Löwenstein Jensen (LJ) slope[12]. A single pyruvate incorporated LJ slope (or other suitable egg-based medium) is recommended to optimise growth of *M. bovis*[12]. Some non-tuberculous mycobacteria may not signal in commercial liquid media, and work is needed to establish an adequate evidence base for when to use liquid culture alone. Solid culture is also needed for some specimen types when a range of incubation temperatures are indicated, for example if from superficial lesions.

Automated culture systems can also be used for sensitivity testing, reducing the time to availability of results to 4 to 12 days after inoculation.

Note: If there is insufficient volume of sample for all investigations, tests should be prioritised following medical microbiological advice (see B 27 - Investigation of cerebrospinal fluid).

To reduce the risk of missed positive cultures, and following the manufacturer's instructions for use, liquid cultures that are ultimately negative on an automated system should be visually inspected for evidence of growth before being discarded.

4.5.3 Culture

All specimens are processed as follows:

1. Prepare bottles according to manufacturer's instructions
2. Inoculate the surface of a pH neutral pyruvate-based Löwenstein Jensen (LJ) slope (or other suitable egg-based medium) with 0.2mL of treated specimen and a liquid culture medium (with an appropriate volume as defined by the manufacturer)
3. Inoculate specimens taken from surface sites, for example skin, to two sets of media, one of which is incubated at 28 to 30°C. Two incubation temperatures should also be used for smear positive bronchial washings, as the mycobacteria which most commonly contaminate bronchoscopes and endoscopes prefer the lower temperature for growth. Bone and joint fluid samples may also need to be incubated at two temperatures to optimise recovery of all NTM species[50]. Consider for example, if the sample is from a limb extremity and/or direct smear positive and tuberculosis is not expected
4. Briefly angle slopes to allow the specimen to inoculate the entire surface. Ensure that the caps are tightly fitted
5. Incubate slopes at 35 to 37°C for 8 weeks, extending to 12 weeks if necessary, reading every week to check for possible acid-fast growth[58]
6. Log automated liquid culture bottles to the incubation system and incubate as instructed by the manufacturer. For small volumes, consider extending the incubation period. Isolation rates for CSF can also be increased by adding a liquid medium, such as Kirchner's or Middlebrook 7H9, to the original container and incubating at 35 to 37˚C; as mycobacteria are known to adhere to the walls of plastic containers. Alternatively, one can flush the original container with the broth from an automated liquid culture tube
7. Store the unused treated deposit in case the samples need to be decontaminated. If not needed for other tests, the entire CSF specimen should be cultured to maximise the recovery rate
8. Confirm the presence of Acid Fast Bacilli (AFB) in positive cultures with the Ziehl-Neelsen or auramine-phenol stain. The former may be preferred due to the greater morphological detail obtained. When following up liquid culture positive bottles, it is recommended that a Gram stain is carried out to enable the detection of possible contamination and/or a blood agar plate set up for the same reason
9. Send aliquots of the confirmed positive cultures (in CE Marked leak proof containers in a sealed plastic bag) to the relevant Reference Laboratory in accordance with the postal and transport regulations[110]
10. Check the bacterial overgrowth at the post-decontamination stage by using purity plates

Note: Automated liquid culture plus conventional solid culture on at least one sample of each suitable specimen type should be set up within one working day of receipt. A

six day service should be provided by laboratories to meet the diagnostic standard recommended in the Department of Health Guidelines[2].

4.5.4 Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) are appropriate as a primary diagnostic method in certain circumstances[1]. For a more detailed discussion, see introduction and the NICE recommendations.

1. Molecular detection for diagnosis of tuberculosis is increasingly becoming standard of care, whenever a clinical diagnosis is being seriously entertained
2. As a minimum, rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum*) should be requested on a suitable primary specimen if there is clinical suspicion of TB disease, and:
   - patient has HIV or
   - rapid information about mycobacterial species would alter the patient's care or
   - the need for a large contact-tracing initiative is being explored
3. In children and young people aged 15 years or younger with suspected pulmonary TB, offer rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum*). Usually only one nucleic acid amplification test will be necessary per specimen type (for example, spontaneous sputum, induced sputum or gastric lavage).
4. In young people aged 16–18 years use the same criteria as in adults to decide whether to request rapid diagnostic nucleic acid amplification tests
5. Clinicians should think about a diagnosis of extrapulmonary TB even if rapid diagnostic tests in, for example, cerebrospinal fluid, pleural fluid or ascitic fluid are negative
6. Offer treatment for TB meningitis if clinical signs and other laboratory findings are consistent with the diagnosis, even if a rapid diagnostic test is negative
7. Ideally, rapid diagnostic nucleic acid amplification tests for rifampicin (and possibly isoniazid resistance) should be undertaken routinely on all AAFB smear positive cases or if M. tuberculosis is detected on a NAAT test directly on sputum.
8. As a minimum, for people with clinically suspected TB, rapid diagnostic nucleic acid amplification tests for rifampicin resistance on primary specimens should be undertaken if a risk assessment for multidrug resistance identifies any of the following risk factors:
   - history of previous TB drug treatment, particularly if there was known to be poor adherence to
   - contact with a known case of multidrug-resistant TB
   - birth or residence in a country in which the World Health Organization reports that a high proportion (5% or more) of new TB cases are multidrug-resistant

All NAATs should be carried out in accordance with manufacturer's instructions.

Suitable clinical samples should ideally be sent for conventional AFB microscopy and culture investigations as outlined above.

Note: It should be noted that if there is not enough specimen volume for PCR and culture, then only culture should be done. All samples, even if PCR positive, should be submitted for culture.

4.5.5 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s) ‡
			Temp °C	Atmos	Time		
All conditions	**Sterile samples:** Blood, All bone marrow aspirates, Wound swab, Pus or Pus swabs*, CSF, Ascites, Joint fluid, Pleural fluid	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				*Mycobacterium* species
		LJ + pyruvate	35 – 37	Air	8 weeks extending to 12 weeks if required[58]	Weekly	
	Non-sterile samples: Sputum, Brochoalveolar lavage/bronchial washings, Gastric washings	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				*Mycobacterium tuberculosis*
		LJ + pyruvate**	35 – 37	Air	8 weeks extending to 12 weeks if required[58]	Weekly	
Genitourinary tuberculosis	Urine	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				*Mycobacterium* species
		LJ + pyruvate	35 – 37	Air	8 weeks extending to 12 weeks if required[58]	Weekly	

Skin infections - ‘fish tank granuloma’ - cutaneous ulcer (or “Buruli” or “Bairnsdale ulcer”	Skin lesions Tissue Biopsy	LJ + pyruvate	28 - 30	Air	8 weeks extending to 12 weeks if required[58]	Weekly	*M. marinum* *M. ulcerans* *M. chelonae*
Cardiac surgery	Post-surgery sites eg sternotomy	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer’s recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				*M.chimaera / M. avium – intracellulare group*
		LJ + pyruvate	35 - 37	Air	8 weeks extending to 12 weeks if required[58]	Weekly	

For these situations, add the following:

Clinical details/ Conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Tuberculous meningitis	CSF and other non-sputum specimens	Kirchner’s liquid media OR Middlebrook 7H9	35 – 37	Air	8 weeks extending to 12 weeks if required[58]	Weekly	*Mycobacterium* species

Clinical details/ Conditions	Specimen	Optional media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Osteomyelitis	Consider for bone and joint fluids eg from extremities and/or direct smear positive	Automated liquid systems*	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer’s recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				Non-tuberculous mycobacteria
		LJ + pyruvate	28 - 30	Air	8 weeks extending to 12 weeks if required[58]	Weekly	
Pulmonary tuberculosis	Smear +ve BAL/washing	LJ + pyruvate	28 – 30	Air	4-6 weeks	Weekly	Rapidly growing mycobacteria
Pulmonary tuberculosis	Sputum Brochoalveolar lavage/bronchial washings Gastric washings	LJ + glycerol **Note:** *M.xenopi* do not grow well on medium with pyruvate preferring glycerol.	42	Air	8 weeks extending to 12 weeks if required[58]	Weekly	*M. xenopi*

* Pus swabs are not the specimen type of choice for mycobacteria. This is because if present, may adhere to the swab rather than be transferred successfully to the culture media[135].

** BCG and *M.xenopi* do not grow well on medium with pyruvate preferring glycerol.

Additional supplements may be required for isolating conspicuously fastidious mycobacterial species.

Note: In certain circumstances NAAT testing is appropriate see section 4.5.4.

4.6 Identification

Most laboratories refer their isolates for identification and susceptibility testing to the reference laboratories.

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: For some NTM isolates, especially rapidly growing mycobacterial (RGM) isolates (*M. fortuitum, M abscessus*, and *M. chelonae*), other identification techniques may be necessary including extended antibiotic in vitro susceptibility testing, DNA sequencing or polymerase chain reaction (PCR) restriction endonuclease assay (PRA). Due to differences in antimicrobial susceptibility that determine treatment options, species-level identification of the NTM is becoming increasingly clinically important. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of an NTM isolate from a normally sterile site such as blood. For initial clinical mycobacterial isolates, however, it is sometimes difficult to determine the clinical significance of the isolate without species identification. Therefore, identification of most mycobacterial isolates to the species level and not merely as groups, such as *M.chelonae/abscessus group* is strongly recommended[50].

In the event that a specific laboratory does not have the necessary technology for species identification of an NTM isolate, the isolate could be sent to a Mycobacterium reference laboratory (see 4.9) for further analysis.

4.6.1 Minimum level of identification in the laboratory

Mycobacterium genus level (based on Ziehl-Neelsen or auramine-phenol stain from culture).

At least one AFB isolate from each new patient should be identified to complex/species level, and suitable susceptibility tests performed if identified as MTBC[2]. Such tests are usually performed at a mycobacterial reference laboratory (listed below).

Repeat AFB isolates from the same patient should usually be identified again and susceptibility tests performed for MTBC, if cultured from a sample collected three months or more after a previously referred MTBC isolate[2].

4.7 Antimicrobial susceptibility testing

Refer to the National Mycobacterium Reference Service (NMRS) as required.

For other devolved national reference regions, refer to section 4.9 for more information.

At least one AFB isolate from each new patient should be identified to complex/species level and suitable susceptibility tests performed if identified as MTBC[2].

Repeat AFB isolates from the same patient should be identified again and susceptibility tests performed for MTBC if cultured from a sample collected three months or more after a previously referred MTBC isolate[2].

4.8 Referral for outbreak investigations

As tuberculosis is a notifiable disease in the UK, for public health management of cases, contacts and outbreaks, all suspected cases should be notified immediately to the local Public Health England Centres.

All clinically significant isolates should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures and all such isolates should be referred to the national reference laboratory for further testing.

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Note: Only send AFB positive isolates. If sending anything else, discuss with the reference laboratory first. Retain an aliquot or culture pending final report.

Isolates for identification and susceptibility testing should be sent to the appropriate RCM within one working day of the culture becoming positive[2]. If the Mycobacteria Growth Indicator Tube (MGIT®) culture system is used, the culture should be incubated for a further 48 hours before despatch to achieve suitable biomass. Isolates should reach the RCM within one working day of despatch[2].

Isolates associated with outbreaks, where epidemiologically indicated, organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem or anomaly that requires elucidation should be sent to the appropriate reference laboratory – if not been sent already.

Advice on rapid culture techniques or other diagnostic services may also be sought from the following:

National Mycobacterium Reference Service South
Public Health England,
National Infection Service,
61 Colindale Avenue,
London
NW9 5HT
Tel: 0208 3276957

National Mycobacterium Reference Service North and Central
Public Health England West Midlands, Birmingham Laboratory
Birmingham Heartlands Hospital
Bordesley Green East
Birmingham
B9 5SS
Tel: 0121 424 3247

Wales Centre for Mycobacteria (WCM)
Public Health Wales, Microbiology Cardiff
Llandough Hospital
Penlan Road
Penarth
CF64 2XX
Tel: 029 2071 6408
http://www.wales.nhs.uk/sites3/page.cfm?orgId=457&pid=25286

Northern Ireland Mycobacterium Reference Laboratory
Department of Microbiology
Kelvin Building
Royal Victoria Hospital
Grosvenor Road
Belfast
BT12 6BA
Tel: 028 9063 4125

Scottish Mycobacteria Reference Laboratory
Clinical Microbiology
Royal Infirmary of Edinburgh
51 Little France
Old Dalkeith Road
Edinburgh
EH16 4SA
Tel: 0131 242 6016

Refer to local Regional Reference Laboratory procedures for strain typing.

5 Reporting procedure

5.1 Microscopy

Report the presence or absence of AFBs.

5.1.1 Microscopy reporting time

Microscopy result should be reported within one working day of receipt of the specimen[2,11].

New positive results should be transmitted to a member of the clinical team responsible for the patient's care[2].

5.2 Culture

Positives

Mycobacterium species isolated (together with comment on potential non-tuberculous mycobacterial identification, if appropriate, according to local protocols).

Negatives

Mycobacterium species not isolated.

5.2.1 Culture reporting time

New culture positives and clinically urgent results: communicate when available.

To meet internationally accepted criteria, mycobacterial samples should be cultured, and acid fast bacilli isolated and identified within 21 days of the source laboratory receiving a specimen for at least 90% of such samples[2].

Issue negative reports from automated liquid system according to manufacturer's instructions.

If solid culture media is used, issue negative report at 8 to 12 weeks.

Molecular

NAATs reporting

Positives

MTBC complex /non -tuberculous mycobacteria (NTM) /MDR-TB detected.

Negatives

MTBC complex / / non -tuberculous mycobacteria (NTM) / MDR-TB not detected.

NAATs reporting time

PCR results are reported as they are done.

Strain typing reporting

The 24 MIRU-VNTR loci are reported by the Mycobacterial Reference Laboratories via Mycobnet (Mycobacterial Surveillance Network) as a 24-digit string in the same order, namely:

- 5 ETRs: A,B,C,D,E ; followed by ten MIRUs: 2,10,16,20,23,24,26,27,39,40; followed by

- 9 VNTRs: 424,1955,2163b,2347,2401,3171,3690,4052,4156

These help minimise false clustering and enable international standardisation.

If a locus contains over 9 repeats, capital letters are used to indicate copy number (A for 10 and so on).

If a partial deletion of a repeat in ETR-D is present, then all the repeats are counted including the partial repeat, and presence of the partial repeat in the count is indicated by a´.

It has been recommended that an MIRU-VNTR genotype for each new MTBC isolate should be available, and entered on the national database, within 21 days of mycobacterial reference laboratory receipt for ≥95% isolates[2].

Note: PHE initially developed an on-line tool to provide a shorthand “cluster name” for identification of a molecular cluster rather than the full 24-digit code but it has been recommended that this is discontinued in order to reduce costs, improve efficiency and increase effectiveness. However, there are currently advancing technologies that will help inform the future of strain typing[147].

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated.

For MTBC, the results of susceptibility testing of primary therapeutic agents (that is, isoniazid, rifampicin, pyrazinamide, ethambutol) should be available within 30 days of receipt of the relevant sample in the source laboratory for ≥95% of such samples[2].

These results should be available within 14 days of MTBC isolate receipt by the relevant sensitivity testing laboratory[2].

6 Notification to PHE[148,149], or equivalent in the devolved administrations[150-153]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare

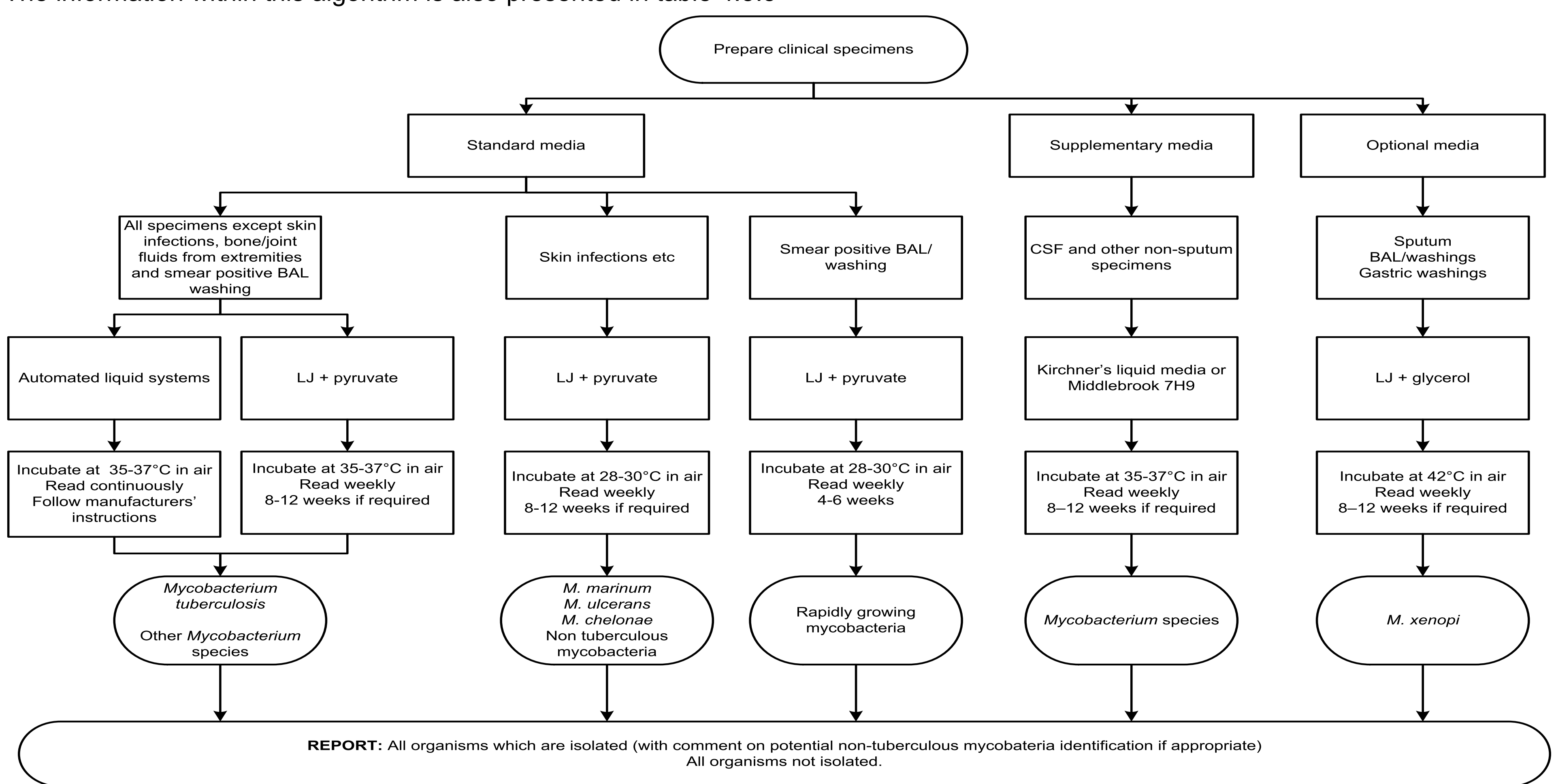
Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[150,151], Wales[152] and Northern Ireland[153].

Appendix: Investigation of specimens for *Mycobacterium* species

The information within this algorithm is also presented in table 4.5.5



In certain circumstances NAAT testing is appropriate see section 4.5.4.

References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

B 40 Investigation of specimens for *Mycobacterium* species

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 13/03/2015 – 13/04/2015

Version of document consulted on: B 40dx+

Proposal for changes

<table>
<tr><td>Comment number</td><td>1</td><td colspan="2"></td></tr>
<tr><td>Date received</td><td>18/03/2015</td><td>Lab name</td><td>Sheffield Teaching Hospitals, Microbiology Laboratory</td></tr>
<tr><td>Section</td><td colspan="3">Various</td></tr>
<tr><td colspan="4">Comment</td></tr>
<tr><td colspan="4">a. 4.5.1.1 Decontamination of specimens using 4% NaOH
i. The decontamination time has changed from 30 minutes in the previous SMI to 15 minutes in the current version. Is there any evidence to support this change?
ii. There is no mention of how much phosphate buffer or water to neutralise with. Equal volume, equal volume of original specimen or fill up the universal.
b. 4.5.1.2 Decontamination of specimens using (0.5N) H_2SO_4
i. If the deposit has already been re-suspended in phosphate buffer is addition of NaOH with phenol red still required? Clarification required.</td></tr>
<tr><td>Recommended action</td><td colspan="3">a.
i. NONE
There are 3 references to support the statement. The decontamination time has been changed because of the harmful effect of NaOH to the tubercle bacilli.
ii. ACCEPT
This has been updated accordingly.
b.
i. NONE
NaOH with phenol red indicator is still added to the deposit already re-suspended in phosphate buffer so that neutralisation can be verified visually.</td></tr>
</table>

Comment number	2		
Date received	19/03/2015	Lab name	Crosshouse Hospital
Section	4.5.1 and 4.5.1.3		
Comment			
Initial list of decontamination methods show 3% oxalic acid but methods later only show 5% oxalic acid.			
Evidence			
5. Decontamination of specimens using oxalic acid (3%) and 4.5.1.3 Decontamination of specimens using 5% oxalic acid[113]			
Recommended action	ACCEPT This has been updated in the document accordingly.		

Comment number	3		
Date received	27/03/2015	Lab name	Medical Microbiology Department, Northern Health and Social Care Trust
Section	Introduction		
Comment			
In the section on non-tuberculous mycobacteria (NTM), it states that they are not transmitted from person to person. However there is evidence of transmission between cystic fibrosis patients.			
Evidence			
https://www.cysticfibrosis.org.uk/media/381091/CC15%20-20NTM%20guidelinesv2.pdf			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	ACCEPT This section of the document has been updated accordingly.		

Comment number	4		
Date received	28/03/2015	**Lab name**	Pathology Laboratory - Cayman Islands
Section	4.5.5		
Comment			
Questioning whether the target organism for pulmonary tuberculosis in the matrix should say *M. tuberculosis* instead of *M. xenopi*.			
Recommended action	**ACCEPT** This has been updated in the document both in section 4.5.5 and the flowchart accordingly.		

Comment number	5		
Date received	08/04/2015	**Lab name**	Public Health Wales Microbiology
Section	Non-tuberculous Mycobacteria		
Comment			
In this section it states “NTM are not transmitted from person-to-person.” Although current (old) guidance still states this is the case, there is growing evidence to suggest this may not be so black and white and I would be wary of making such a definite statement. I am thinking of work performed in Cambridge and Seattle, references below.			
Evidence			
1. Bryant JM, Grogono DM, Greaves D, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. Lancet 2013; published online March 29. http://dx.doi.org/10.1016/S0140-6736(13)60632-7. 2. Aitken ML, Limaye A, Pottinger P, et al. Respiratory outbreak of *Mycobacterium abscessus* subspecies *massiliense* in a lung transplant and cystic fibrosis center. Am J Respir Crit Care Med 2012; 185: 231-32.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** This section of the document has been updated accordingly. The references have been used to solidify the evidence that NTM (only *M. abscessus* so far) can be transmitted from person		

	to person.

Comment number	6		
Date received	10/04/2015	Lab name	Truro
Section	Pages 17, 18, 21, 26, 30, 32 and 40		
Comment			
a. Pg 17 Quality Control section needs a change in font size to match the rest of the document. b. Pg 18 1.2 Paragraph 7 - Air flow readings must be taken with the hot plate off - Please provide references and evidence. May not be applicable for Class 1 cabinet. c. Pg 21 Blood section - This SOP refers to B37 for Blood cultures but B37 refers to B40 (old MAI SOP is merged into TB SOP not blood cultures) d. Pg 26 - Decontamination of specimens - We decontaminate for 25 mins (Newcastle method) e. Pg 30 4.5.4 Point 2 - Not clear what the role is in TBM f. Pg 32 - Pulmonary tuberculosis incubation temperature is not routine at 42C g. Pg 40 - This flowchart does not include a section on *M. xenopi* at 42C shown on 4.5.5 table - this should be an option if required.			
Recommended action	a. **ACCEPT** Font size has been increased in line with the UK SMI template. b. **NONE** This is recommended as good practice. This reference (British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14) discusses the use of appropriate equipment in the MSC but not specifically on the use of hot plates. This was discussed at the Bacteriology Working Group meeting and a form of words has been agreed and this has been updated in the document. c. **NONE** This section refers to B 37 for further information on collection and processing of blood cultures while in B 37 document; users are referred to B 40 to use this document when investigating specimens for *Mycobacterium* species. d. **ACCEPT**		

	This has been updated and references added accordingly. e. **NONE** This information is already covered in the document. f. **ACCEPT** This has been moved to the optional section in table 4.5.5 if needed. g. **ACCEPT** The flowchart has been amended to include the optional section on *M. xenopi* if needed.

Comment number	7		
Date received	13/04/2015	**Lab name**	Public Health Wales
Section	Various		
Comment			

a. P8 “Introduction”.

For a complete list of MTBC members, there is at least one novel species, *M. mungi* from Alexander KA, Laver PN, Michel AL, Williams M, van Helden PD, Warren RM, Gey van Pittius NC. Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. Emerg Infect Dis. 2010 Aug;16(8):1296-9. doi: 10.3201/eid1608.100314. Erratum in: Emerg Infect Dis. 2010 Dec;16(12):2024.

b. P9 “Pulmonary Tuberculosis.

Perhaps the requirement for two or three specimens needs clarification here and later on on P19?

c. P12 - *Mycobacterium avium intracellulare* group (MAI):

There is also *M. chimaera* within the MAI group?

d. P16 - MALDI-TOF Identification of *Mycobacterium* species

MALDI-TOF is now widely available.

e. P19: 2.2.1 Correct specimen type and method of collection Sputum specimens

Clarification needed on 2/3 specimens within the UK/globally?

f. P30 - 4.5.4 Nucleic acid amplification tests.

Perhaps it should be emphasised that if there is not enough specimen volume for PCR and culture, then only culture should be done. Also, that PCR positive specimens should ideally be subsequently confirmed by a corresponding positive culture.

g. P32 - 4.6 Identification

Is there an individual SMI for ID of *Mycobacterium* species?

<table>
<tr><td colspan="2">

h. P32 4.9 Referral to Reference Laboratories

This link is specifically for PHE NMRL. For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms. England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

This PHE link needs clarification as the link for the Wales Centre for Mycobacteria is: http://www.wales.nhs.uk/sites3/page.cfm?orgId=457&pid=25286.

The current address for the Welsh TB lab is: Wales Centre for Mycobacteria (WCM) Public Health Wales Microbiology Cardiff Llandough Hospital Penlan Road Penarth CF64 2XX Tel: 029 2071 6408

i. 5.4 Strain typing reporting

MycoNet is now ETS STM?

j. P38 References

i. Is reference 4 still valid in light of the 2015 PHE strategy?

ii. Should this be included as a reference? Centre for Disease Prevention and Control. Mastering the basics of TB control: Development of a handbook on TB diagnostic methods. Stockholm: ECDC; 2011.

</td></tr>
<tr><td colspan="2">Financial barriers</td></tr>
<tr><td colspan="2">No.</td></tr>
<tr><td colspan="2">Health benefits</td></tr>
<tr><td colspan="2">No.</td></tr>
<tr><td>Recommended action</td><td>

a. **NONE**

This reference will not be accepted because *M. mungi* according to Euzeby, JP 2015 does not have standing in nomenclature and so is not included in the 169 species of *Mycobacterium*.

b. **ACCEPT**

This has been updated with references and reasons as to why the WHO has recommended that at least 2 samples should be processed in a TB case.

c. **ACCEPT**

This has been updated accordingly.

d. **NONE**

MALDI-TOF MS is used but it is not yet widely available in many clinical laboratories due to costs and lack of expertise.

e. **ACCEPT**

See comment b.

</td></tr>
</table>

	f. **ACCEPT** This has been updated in section 4.5.4. g. **NONE** There is no proposal for an individual SMI for Identification of *Mycobacterium* species. Isolates should be sent to appropriate Reference laboratory for further identification. h. **ACCEPT** The link has been added to the Welsh TB lab and address amended. i. **NONE** MycobNet is the correct name and not MycoNet. j. i. **NONE** The validity of the reference in light of PHE strategy remains unclear at this stage. *Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci U S A 2002; 99:3684-9.* ii. **ACCEPT** This reference has been added in the appropriate sections of the document.

Comment number	8		
Date received	13/04/2015	**Professional body**	IBMS
Section	Various		
Comment			
a. Safety considerations in performing MALDI-TOF analysis for the identification of any mycobacteria strains may be worth considering. b. Report time of auramine or ZN slide should be in line with Gram strain. Reporting an AFB microscopy is suggested to be within the next working day whereas a Gram strain, on a CSF for example (SMI B27) must be verbally reported within 2hrs with a hard copy available within 24hrs. c. There is the safety briefing circulated very recently by PHE regarding *M. chimaera* which should also be considered for inclusion in the policy. This is obviously related to a risk of post-operative infections following use of by-pass machines (see attached). The organism belongs to the *M. avium* complex however it would seem sensible to mention it as a separate species and list the new risks related to cardio surgery.			

d.

i. In the introduction it states "The genus *Mycobacterium* is a member of the family Mycobacteriaceae and consists of 168 species and 10 subspecies of which a few have been reclassified to other genera within the family" Ref 3. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Mycobacterium*.

Comment: According to ref 3 there are 170 species and 13 sub species yet this SMI states 168 and 10 sub species. This requires some clarification to avoid confusion.

ii. In addition, within ref 3 states "Note: In 2009, Leao et al. proposed the union of ¤ *Mycobacterium bolletii* and *Mycobacterium massiliense*, and the recognition of two subspecies within *Mycobacterium abscessus*: *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *massiliense*. The proposal of *Mycobacterium abscessus* subsp. *massiliense* was not in accordance with the Rules of the Bacteriological Code, because the epithet *bolletii* has priority over the epithet *massiliense*. Consequently, the name *Mycobacterium abscessus* subsp. *massiliense* was illegitimate. In 2011 Leao et al. propose the correct name *Mycobacterium abscessus* subsp. *bolletii* (Adékambi et al. 2006) Leao et al. 2011."

Comment: There are several publications that do not agree that *M abscessus* subsp *massiliense* is an illegitimate name, see references below:

a) Comparing *Mycobacterium massiliense* and *Mycobacterium abscessus* lung infections in cystic fibrosis patients Journal of Cystic Fibrosis 2015 Jan;14(1):63-9 Anne-Laure Roux et al.

b) Molecular Fingerprinting of *Mycobacterium abscessus* Strains in a Cohort of Pediatric Cystic Fibrosis Patients J. Clin. Microbiol. 2012, 50(5):1758. Kathryn A. Harris,a Dervla T. D. Kenna,b Cornelis Blauwendraat,a John C. Hartley,a Jane F. Turton,b Paul Aurora,cand Garth L. J. Dixona Department of Microbiology, Virology and Infection Control, Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom[a]; Laboratory for Healthcare Associated Infection, HPA Centre for Infections, London, United Kingdom[b]; and Paediatric Respiratory Medicine and Lung Transplantation, Great Ormond Street Hospital.

c) Cohort Study of Molecular Identification and Typing of *Mycobacterium abscessus, Mycobacterium massiliense*, and *Mycobacterium bolletii* JOURNAL OF CLINICAL MICROBIOLOGY, July 2009, p. 1985–1995 Vol. 47, No. 7 Adrian M. Zelazny,[1,2*] Jeremy M. Root,[2] Yvonne R. Shea,[1] Rhonda E. Colombo,[2] Isdore C. Shamputa,[3]Frida Stock,[1] Sean Conlan,[4] Steven McNulty,[5] Barbara A. Brown-Elliott,[5] Richard J. Wallace, Jr.,[5]Kenneth N. Olivier,[2] Steven M. Holland,[2] and Elizabeth P. Sampaio.

e. In the section Non-Tuberculous Mycobacteria (NTM) it states " NTM are ubiquitous in nature, have a varied spectrum of pathogenicity for humans, are not transmitted from person to person and are often resistant to classical anti-tuberculous chemotherapy[41,42].

Comment: While this may be true of most NTM, there are several publications confirming the transmission of *M abscessus* complex, in particular *M massiliense* in the CF population but also in other clinical settings: see references below.

d) The growing threat of non-tuberculous mycobacteria in CF Journal of Cystic Fibrosis (2014) Volume 14, Issue 1, Pages 1–2 R. Andres Floto * Charles S

Haworth

e) Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study: Lancet 2013; 381: 1551–60 R Andres Floto.

f) Molecular Characterization of *Mycobacterium massiliense* and *Mycobacterium bolletii* in Isolates Collected from Outbreaks of Infections after Laparoscopic Surgeries and Cosmetic Procedures_ J CLIN MICRO, March 2008, p. 850–855 Vol. 46, No. 3 Cristina Viana-Niero,1 Karla Vale´ria Batista Lima,2 Maria Luiza Lopes,

g) Phenotypic and molecular characterization of quinolone resistance in *Mycobacterium abscessus* subsp. *bolletii* recovered from postsurgical infections. J Med Microbiol. 2012 Jan;61(Pt 1):115-25.de Moura VC1, da Silva MG, Gomes KM, Coelho FS, Sampaio JL, Mello FC, Lourenço MC, Amorim Ede L, Duarte RS.

h) Respiratory Outbreak of *Mycobacterium abscessus* Subspecies *massiliense* in a Lung Transplant and Cystic Fibrosis Center

To the Editor: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE VOL 185 2012 Moira L. Aitken, M.D.

f. NTM Identification section 4.6 states “Refer to individual SMIs for organism identification. Organisms may be further identified if this is clinically or epidemiologically indicated”

Comments: As stated within ref 41

“ For some NTM isolates, especially rapidly growing mycobacterial (RGM) isolates (*M. fortuitum, M abscessus*, and *M. chelonae*), other identification techniques may be necessary including extended antibiotic in vitro susceptibility testing, DNA sequencing or polymerase chain reaction (PCR) restriction endonuclease assay (PRA).”

“Because of differences in antimicrobial susceptibility that determine treatment options, species-level identification of the NTM is becoming increasingly clinically important. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of an NTM isolate from a normally sterile site such as blood. For initial clinical mycobacterial isolates, however, it is sometimes difficult to determine the clinical significance of the isolate without species identification. Therefore, identification of most mycobacterial isolates to the species level and not merely as groups, such as “*M. chelonae/abscessus group*” is strongly recommended. If, after consultation between the clinician and the laboratorian and in the event that a specific laboratory does not have the necessary technology for species identification of an NTM isolate, the isolate could be sent to a reference laboratory for further analysis.”

g. Why does this SMI document refer to MALDI-TOF for identification of *Mycobacterium* spp that has not been fully validated yet does not discuss the standard HAIN assay that is currently in use for the identification of NTM, or its limitations (see ref i below and ref 111)? With reference to NTM in particular the *abscessus* complex, HAIN is unable to separate the members of the complex and is known to misidentify some strains as *M chelonae*.

i) Comparison of two methods for identification of *Mycobacterium abscessus* and

	Mycobacterium chelonae by K.M. Sands, A. Nicholson, C. Rennison, A. Barrett, S. Bourke, A. Robb, K. Gould, J.G. Magee Journal of Cystic Fibrosis (Vol.11) Volume 11, Supplement 1 , Page S85, June 2012. http://www.cysticfibrosisjournal.com/article/S1569-1993%2812%2960284-7/abstract?source=aemf h. In addition there is no recommendation to refer these isolates to a specialist reference laboratory that uses molecular identification methods e.g Colindale deploys sequencing of housekeeping genes rpoB, hsp65, sodA, nor does it recommend strain typing of the *M abscessus* complex. i. Whilst this document quotes there is no evidence of person to person spread of NTM, without typing how do you know? Colindale are working toward WGS but currently strain type *M abscessus* complex using VNTR sequence cluster analysis. This is an important point especially with respect to CF isolates as without proper identification and strain typing we will not be able to monitor *M abscessus* complex effectively. See refs d, e, f, g and h. j. Rapid Growing species States "*M. abscessus* more so than the other non-tuberculous mycobacterium are an increasing problem for the cystic fibrosis patient group 49. Testing should be considered in patients who show deteriorating lung function but where no clear pathogen has been identified 50-52". Comments: The CF trust recommends annual screening of CF sputum for NTM, we would query why.
Recommended action	a. **ACCEPT** This has been updated in the document accordingly. b. **NONE** This has not been recommended and so Gram stain and auramine or ZN slides should be reported as stated in the various SMIs. c. **ACCEPT** This has been updated in the document accordingly. d. i. **ACCEPT** The update on the taxonomy of the genus *Mycobacterium* has been made and the BWG members agreed that it should be stated that the genus *Mycobacterium* consists of over 100 species because of the continuous taxonomy update. ii. **NONE** The UK SMIs follow the approved list of bacterial names laid out in Rules of the Bacteriological Code. e. **ACCEPT** This section of the document has been updated accordingly. Some of the references have been used to prove that transmission of NTM (*M. abscessus*) between

	patients is possible. f. **ACCEPT** This section of the document has been updated accordingly. g. **ACCEPT** The standard HAIN assay has been updated in the document and its limitations are mentioned in the technical limitations. However, MALDI-TOF MS is mentioned as it is very useful in the identification for this important group of pathogens, potentially allowing accurate treatment regimens to be started earlier, although it is not widely available yet in all clinical laboratories due to lack of expertise and costs. h. **NONE** All suspected/confirmed *Mycobacterium s*amples are referred to reference laboratories. i. **ACCEPT** See comment 5. j. **NONE** We are not in a position to comment on another guideline producer's recommendation.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	30/03/2015	**Lab name**	Hairmyres Hospital Microbiology Laboratory

UK Standards for Microbiology Investigations

Investigation of urine

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 41 | Issue no: 8.7 | Issue date: 11.01.19 | Page: 1 of 51

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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 7

Scope of document 9

Introduction 9

Technical information/limitations 21

1 Safety considerations 24

2 Specimen collection 24

3 Specimen transport and storage 26

4 Specimen processing/procedure 27

5 Reporting procedure 36

6 Notification to PHE, or equivalent in the devolved administrations 38

Appendix 1: Multiplicative factors based on varying volumes of urine dispensed, diameter of well & field of vision (Fov) diameter 39

Appendix 2: Multiplicative factor equation 40

Appendix 3: Guidance for the interpretation of urine culture 41

Appendix 3 (continued): Guidance for the interpretation of urine culture 42

References 43

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	18/11.01.19
Issue no. discarded.	8.6
Insert issue no.	8.7
Section(s) involved	**Amendment**
Section 4.7.1 Antimicrobial susceptibility testing and reporting table	A comment was added for Tazobactam which should not be tested or reported for *Acinetobacter spp.*

Amendment no/date.	17/07.08.18
Issue no. discarded.	8.5
Insert issue no.	8.6
Section(s) involved	**Amendment**
Section 2.2 Optimal time and method of collection.	Removed recommendation for periurethral cleaning in mid-stream urine (MSU) collection.

Amendment no/date.	16/01.05.18
Issue no. discarded.	8.4
Insert issue no.	8.5
Section(s) involved	**Amendment**
Section 5.2 Culture.	Typo amended in text.

Amendment no/date.	15/16.08.17
Issue no. discarded.	8.3
Insert issue no.	8.4
Section(s) involved	**Amendment**

Page 19 Interpretation of culture.	Bacteria levels have been corrected.

Amendment no/date.	14/07.08.17
Issue no. discarded.	8.2
Insert issue no.	8.3
Section(s) involved	**Amendment**
Page 14 Organisms implicated in UTI.	Spelling error corrected.
Page 14 Organisms implicated in UTI.	Reorganisation of some text.

Amendment no/date.	13/06.06.17
Issue no. discarded.	8.1
Insert issue no.	8.2
Section(s) involved	**Amendment**
Introduction.	Definition of pyuria added and referenced.
Appendix 2.	Formula and definitions updated.

Amendment no/date.	12/06.09.16
Issue no. discarded.	8
Insert issue no.	8.1
Section(s) involved	**Amendment**
Types of urine specimen and collection.	Spelling error corrected.

Amendment no/date.	11/15.08.16
Issue no. discarded.	7.2
Insert issue no.	8
Section(s) involved	**Amendment**

Introduction.	This has been expanded to include pyuria, haematuria, and Candida species. The section has been streamlined to make it easier to find information that relates to men and women. Healthcare associated UTI has now been changed to 'Catheterisation'. The section on semi-automated methods has been expanded to include more systems. Legionella urinary antigen detection has been added to the document. Screening for *Neisseria gonorrhoea* has been added.
Technical information/limitations.	"Transport of urine specimens" section has been expanded. Section on "Validation and verification" has been added. Section on "Carry over contamination" has been added.
Antimicrobial susceptibility testing.	This section has been expanded and now includes a reporting table.
Reporting procedure.	Two new sections have been added to this; "Microscopy or chemical screening reporting times" and "Urine for antigen testing". Note for *T. hominis* removed.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratorieshttp://www.hpa-standardmethods.org.uk/. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user’s risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Bag urine, pad urine, catheter urine, prostate massage/secretions, clean catch urine, suprapubic aspirate, cystoscopy urine, ureteric urine, ileal conduit urine, urostomy urine, mid-stream urine, nephrostomy urine

This SMI describes the processing and bacteriological investigation of urine samples. These include mid-stream and clean catch specimens and those collected via bag, ileal conduit, ureter, catheter, urostomy, nephrostomy, cystoscopy, supra pubic aspirate, prostate massage/secretions, and pad urine.

This SMI does not describe in detail semi-automated systems such as urine analysers which should be validated and used in accordance with manufacturers' instructions. Due regard should be taken of various groups including pregnant women, children, men and all patients who are immunocompromised.

This SMI also covers the detection of Legionella urinary antigens.

This SMI should be used in conjunction with other SMIs.

Introduction

Urinary tract infection

Urinary tract infection (UTI) results from the presence and multiplication of microorganisms, in one or more structures of the urinary tract, with associated tissue invasion[1]. This can give rise to a wide variety of clinical syndromes. These include acute and chronic pyelonephritis (kidney and renal pelvis), cystitis (bladder), urethritis (urethra), epididymitis (epididymis) and prostatitis (prostate gland). Infection may spread to surrounding tissues (eg perinephric abscess) or to the bloodstream.

Protection against infection is normally given by the constant flow of urine and regular bladder emptying. Urine is a poor culture medium for many bacteria due to its acidity, high urea concentration and variable osmolality and, in men, possibly partly as a result of antibacterial activity of prostatic secretions[2].

The following is a list of terms used in UTI:

Bacteriuria[3]

Bacteriuria implies that bacteria are present and may be cultured from urine. The patient may or may not be symptomatic.

Pyuria is defined as the presence of 10 or more white blood cells per cubic millimetre in a urine specimen, 3 or more white cells per high-power field of unspun urine, a positive result on Gram's staining of an unspun urine specimen, or a urinary dipstick test that is positive for leucocyte esterase[4]. It is most commonly associated with a bacterial urinary tract infection in the upper or lower urinary tract. Pyuria may be present in septic patients, or in older patients with pneumonia[5].

Other conditions that could cause pyuria are infections (such as that caused by *C. trachomatis*, *N. gonorrhoeae*, or herpes simplex virus and occasionally in women with vaginitis caused by *T. vaginalis* or candida infections), pyelonephritis, papillary necrosis, diabetes, renal tuberculosis, renal stones, Kawasaki disease and cancer[6].

Sterile Pyuria (ie no growth on routine culture media and the persistent presence of white blood cells in the urine) may be the result of many factors including: a result of prior treatment with antimicrobial agents; catheterisation; calculi (stones); or bladder neoplasms. Other conditions which may lead to sterile pyuria include genital tract infection; sexually transmitted diseases, eg *C. trachomatis* or an infection with a fastidious organism[7,8]. Renal tuberculosis may also be implicated in sterile pyuria but is uncommon, although should be considered if clinically indicated (eg in high risk populations)[9].

Haematuria[10] **–** Haematuria is observed in patients with acute cystitis, but is rarely seen in association with other dysuric syndromes. Finding 1–2 red blood cells (RBCs)/high power field is not considered to be abnormal. Haematuria may be caused by non-infective pathological conditions of the urinary tract or by renal mycobacterial infection, with or without associated pyuria. Apparent haematuria may be the result of menstruation. Differentiation of dysmorphic RBCs to determine those of glomerular origin is sometimes requested by specialist units, although its reliability is disputed[11,12]. RBC lysis may occur in hypertonic and hypotonic urine, rendering them undetectable by microscopy.

Symptomatic patients

Symptomatic patients may be bacteriuric or abacteriuric. Symptoms in children and the elderly, when present, may be non-specific and difficult to interpret.

Frequency

The average bladder capacity is about 500mL. Significant reduction in capacity accompanies acute inflammation which can lead to an increase in the frequency of micturition.

Dysuria

Dysuria is painful and difficult micturition.

Urgency

Urgency is a strong desire to empty the bladder, which can lead to incontinence.

Nocturia

Nocturia is waking in the night one or more times to void the bladder[13,14]. Nocturnal enuresis is the involuntary voiding of urine during sleep, ie bed-wetting.

Incontinence

Incontinence is the involuntary leakage of urine. The commonest form of this is stress incontinence where leakage accompanies an increase in intra-abdominal pressure due to sneezing, coughing or laughing. Overflow or dribbling incontinence accompanies an overfilled bladder.

Renal colic

This is characterised by very severe cramping pain resulting from distension of the ureter and pelvis above an obstruction such as a renal stone. Often accompanied by frequency and urgency.

Clinical manifestations of UTI

Asymptomatic bacteriuria and candiduria

Asymptomatic bacteriuria is common in several patient groups, particularly the elderly, pregnant women, transplant patients and diabetic patients[15-17].

Acute urethral syndrome

Acute urethral syndrome occurs in women with acute lower urinary tract symptoms with either a low bacterial count or without demonstrable bacteriuria or vulvovaginal infection[7,18]. The condition can also occur in men but is not well studied[19].

Uncomplicated UTI

Uncomplicated UTI occurs in otherwise healthy individuals. There are no underlying structural or neurological lesions of the urinary tract, and no other systemic diseases predisposing the host to bacterial infection. Recurrences are usually reinfections with organisms ascending via the urethra.

Acute uncomplicated cystitis

Acute uncomplicated cystitis condition usually occurs in young women. It has an abrupt onset and produces severe symptoms which are usually accompanied by pyuria and bacteriuria. Uncomplicated cystitis can occur in some men[20].

Complicated UTI

Complicated UTI occurs in patients in whom there may be residual inflammatory changes following recurrent infection or instrumentation, obstruction, stones, or anatomical or physiological abnormalities or pathological lesions. These interfere with drainage of urine in part of the tract which encourages prolonged colonisation. Relapses with the same organism may occur.

The following are examples of complicated UTI:

Acute pyelonephritis (pyelitis) – An inflammatory process of the kidneys and adjacent structures. Symptoms include loin, low back or abdominal pain and fever. Symptoms of cystitis may also be present. Severity ranges from mild disease to full blown Gram negative sepsis.

Chronic pyelonephritis (chronic interstitial nephritis, or reflux nephropathy) **–** Controversy exists over the definition and cause of this syndrome. It is the second most common cause of end-stage renal failure. It is thought to be a result of renal damage caused by UTI in infants and children with vesicoureteric reflux, or by obstructive uropathy in adults. However, it is still unclear whether recurrent infection causes progressive kidney damage.

Perinephric abscess – A complication of UTI, although uncommon, that affects patients with one or more anatomical or physiological abnormalities. The abscess may be confined to the perinephric space or extend into adjacent structures. Pyuria, with or without positive culture, is seen on examination of urine, but is not always present. Causative organisms are usually Gram negative bacilli, but can also be staphylococci or *Candida* species. Mixed infections have also been reported.

Prostatitis[21,22] – An inflammatory condition of the prostate gland that occurs in a variety of different forms, some involving infection. Routes of infection of the prostate include ascending urethral infection, reflux of infected urine into the prostatic ducts

that empty into the posterior urethra, invasion of rectal bacteria by direct extension, or by lymphatic or haematogenous spread.

Types of prostatitis include:

- **acute bacterial prostatitis –** An abrupt, febrile illness with marked constitutional and genitourinary symptoms.
- **chronic bacterial prostatitis –** Relapsing and recurrent UTIs, caused by the organisms persisting in the prostatic secretions despite antimicrobial therapy. The method of Meares and Stamey compares white blood cell (WBC) and bacterial counts of urethral, mid-stream and post-prostatic massage urine specimens, and expressed prostatic secretions (EPS)[23]. Prostatic massage should not be undertaken in patients with acute prostatitis because of the risk of precipitating bacteraemia. All specimens are taken at the same time and processed immediately[23]. Chronic bacterial prostatitis is less common than non-bacterial prostatitis. Bacterial prostatitis is associated with UTI. Organisms responsible are similar to those that cause UTI.

Pyonephrosis – The bacterial infection of an obstructed ureter which fills with pus. This may follow surgical intervention. Diagnosis is made from blood culture or pus drained from the kidney.

Renal abscesses – Localised in the renal cortex and may occur as a result of bacteraemia. Pyuria may also be present, but urine culture is usually negative. Renal abscesses are increasingly being seen as complications of acute pyelonephritis caused by Gram negative bacilli. The rare condition of emphysematous pyelonephritis, which results in multifocal intrarenal abscesses and gas formation within the renal parenchyma, is usually seen in diabetic patients or as a complication of renal stones. *Escherichia coli* is the commonest cause.

Urethritis – Common in both male and female patients, and is often associated with UTI or occasionally with bacterial prostatitis.

In men, urethritis is commonly caused by sexually transmitted diseases and is associated with urethral discharge. The main organisms responsible are: *Neisseria gonorrhoeae* (gonococcal urethritis), *Chlamydia trachomatis* and *Mycoplasma genitalium* (non-gonococcal urethritis or NGU).

In female patients the condition may appear as acute urethral syndrome or urethrocystitis caused by Enterobacteriaceae, *Staphylococcus saprophyticus,* and less commonly by *C. trachomatis* and *N. gonorrhoeae*.

Incidence of UTI

The incidence of UTIs is influenced by age, sex or by predisposing factors that may impair the wide variety of normal host defence mechanisms[2].

Children

UTI is a common bacterial infection that causes illness in children, in whom it may be difficult to diagnose as the presenting symptoms are often non-specific[24,25]. In children, the condition is often associated with renal tract abnormalities and is most common in males in the first three months of life as a result of congenital abnormalities. In older children, females are more commonly affected. Infection in pre-school boys is often associated with renal tract abnormality. Failure to diagnose and treat UTI quickly and effectively may result in renal scarring and ultimately loss of

function. The phenomenon of vesicoureteric reflux, while predisposing children to UTI, may also be caused by UTI[26-29].

Confirmation of UTI in children is dependent on the quality of the specimen, which is often difficult to obtain cleanly. The probability of UTI is increased by the isolation of the same organism from two specimens.

Colony counts of $\geq 10^6$ cfu/L (10^3 cfu/mL) of a single species may be diagnostic of UTI in voided urine. Generally, a pure growth of between 10^7-10^8 cfu/L (10^4-10^5 cfu/mL) is indicative of UTI in a carefully taken specimen.

Negative cultures or growth of $<10^7$ cfu/L ($<10^4$ cfu/mL) from bag urine may be diagnostically useful. Counts of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) should be confirmed by culture of a more reliable specimen, either a single urethral catheter specimen or, preferably, an SPA.

Bacteriuria usually exceeds $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) in SPAs from children with acute UTI, although any growth is potentially significant.

Adults

Women

The incidence of UTI is highest in young women. Around 10–20% of women will experience a symptomatic UTI at some time. In acutely symptomatic women, UTI may be associated with counts of a single isolate as low as 10^5 cfu/L (10^2 cfu/mL) in voided urine[30,31]. Interpretation of culture results must be made with care however, and take into account factors such as age and storage of specimen, level of contamination indicated by SECs, and the sensitivity of the method.

Growths of $<10^8$ cfu/L ($<10^5$ cfu/mL) in asymptomatic, non-pregnant women are rarely persistent and usually represent contamination.

Men

Most infections in adult men are complicated and related to abnormalities of the urinary tract, although a low incidence occurs spontaneously in otherwise healthy young men.

Counts as low as 10^6 cfu/L (10^3 cfu/mL) of a pure or predominant organism have been shown to be significant in voided urine from men[32]. Where there is evidence of contamination, a carefully collected repeat specimen should be examined.

Diagnosis of prostatitis may be achieved by comparing the levels of pyuria in sequential specimens taken in association with prostatic massage[23]. If the level of pyuria after prostatic massage is 10 times that of the initial urine, then bacterial prostatitis is likely. More than 15 WBCs per high power field in expressed prostatic secretions is considered abnormal, even if the WBCs in the urethral and bladder urine are within the normal range.

The elderly

UTI incidence increases with age for both sexes and is one of the most common infections associated with this age group[33-35]. It is estimated that 10% of males and 20% of females over the age of 80 have asymptomatic bacteriuria[36]. Underlying health issues can make this condition particularly difficult to diagnosis and prone to resistant strains. According to some, no treatment is indicated for asymptomatic patients except before invasive genitourinary procedures.

Pregnancy

Studies in the UK have shown that asymptomatic bacteriuria (persistent colonisation of the urinary tract without urinary symptoms) occurs in 4% of pregnant women[37]. Unless detected and treated early, there is an increased risk of preterm birth and pyelonephritis affecting maternal and fetal outcome. In about 30% of patients acute pyelonephritis occurs, especially at the time of delivery[38,39]. It has been reported that 20–40% of pregnant women with untreated bacteriuria will develop pyelonephritis[38].

In pregnancy, routine and sensitive urinary screening programmes are essential for the detection of bacteriuria in pregnancy. The screening can be done by mid-stream urine culture early in pregnancy. The presence of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) in asymptomatic, pregnant women indicates infection but should be confirmed in a repeat sample[40].

Diabetes

Women with diabetes have a higher incidence of asymptomatic bacteriuria than those without[41,42]. There is no difference in the prevalence of bacteriuria between men with diabetes, and men without diabetes[42]. There is a debate as to whether factors such as glycosuria, age or instrumentation are contributory to the high prevalence of UTI, but bladder dysfunction as a result of diabetic neuropathy may be the major predisposing factor[41]. The relative incidences of symptomatic infection in patients with or without diabetes remain unclear but, when they do occur, UTIs tend to be more severe in patients with diabetes[43,44].

Neuromuscular disorders

Patients with impaired bladder innervation as a result of congenital or acquired disorders (spina bifida, spinal cord injury) are at increased risk of UTI and it can be a significant cause of death[45]. This may be due to impaired function of the bladder leading to incomplete emptying, or an increased requirement for instrumentation of the urinary tract to assist voiding.

Renal transplantation[46-48]

Most infections occur soon after transplantation, usually as a result of catheterisation, the presence of a ureteric drainage tube, or a previous UTI whilst on dialysis. Less commonly, infection may be introduced via the donor kidney.

Immunosuppression

Overall the incidence of UTI is not higher in patients who are immunocompromised compared with those who are not. The exceptions to this include patients who are diabetic or have undergone renal transplants[49]. There have also been studies that suggest that men who are suffering from acquired immunodeficiency syndrome (AIDS) may also be at increased risk from bacteruria, and symptomatic UTI; with severe episodes resulting in bloodstream infection and death being reported[50]. However, because of long-term antibiotic use for other infections, UTI in such patients is often due to more unusual or resistant organisms. Steroid treatment may induce reactivation of tuberculosis of the urinary tract.

Catheterisation

Catheter acquired urinary tract infections is one of the most common health care acquired infections[51]. However samples from patients with indwelling catheters may

not accurately reflect the true bladder pathogen and often contains several bacterial species. Culture results should be interpreted with caution. The criteria have not been established for differentiating asymptomatic colonisation of the urinary tract from symptomatic infection[52]. Urine cultures may not reflect bladder bacteriuria because sampled organisms may have arisen from biofilms on the inner surface of the catheter[53]. Therefore the quality of the specimen collected and clinical circumstances in the individual patient are critical in the interpretation of bacterial counts. In carefully collected specimens, taken under controlled study conditions in short term catheterised patients, counts of $<10^8$ cfu/L ($<10^5$ cfu/mL) have been shown to be significant[54]. In specimens of unknown quality and those from long term-catheterised patients, interpretation of significance on the basis of bacterial counts alone may be impossible. Significance of isolates and reporting of sensitivities may be indicated in certain groups – such as urology or post-operative patients, especially if future operative intervention is planned on the urinary tract.

Bacterial counts from catheterised patients may be affected by the administration of medication or fluids that increase urine flow, rapid transit of urine from the catheterised bladder, or colonisation with relatively slow growing organisms such as *Candida* species[55].

Catheterisation is occasionally used to collect a contamination free sample ('in and out') when any bacterial growth is significant. Specimens from patients with intermittent self-catheterisation should be treated as mid-stream urine.

Organisms implicated in UTI

Acute, uncomplicated UTIs

Acute, uncomplicated UTIs are usually caused by a single bacterial species.

E. coli – is the most common organism involved in UTI. An international survey of mid-stream urine (MSU) samples taken at 252 centres in 17 countries reports that *E. coli* accounts for 77% of isolates[56].

Only a few serotypes frequently cause UTI. This might reflect their prevalence in the faecal flora, or reflect differences in virulence factors. Certain virulence factors specifically favour the development of pyelonephritis, whereas others favour cystitis or asymptomatic bacteriuria[57].

Proteus mirabilis – Common in young boys and males, and is associated with renal tract abnormalities, particularly calculi. In hospital patients it may cause chronic infections.

S. saprophyticus – Studies have shown that this organism was found to be responsible for 4% of UTIs. *S. saprophyticus* adheres to uroepithelial cells significantly better than *S. aureus* or other coagulase negative staphylococci.

Other coagulase negative staphylococci – Often considered as urinary contaminants as they are part of the normal perineal flora.

Streptococci – Rarely cause uncomplicated UTI, although Lancefield Group B streptococci may cause infection in some women. Enterococci may occasionally cause uncomplicated UTI.

***Candida* species** - is associated with indwelling catheters, but may also be present as contamination from the genital tract. *Candida albicans* is the most frequently isolated species.

Complicated UTIs

Complicated UTIs which occur in the abnormal or catheterised urinary tract are caused by a variety of organisms, many of them with increased antimicrobial resistance as a result of the prolonged use of antibiotics.

E. coli remains the most common isolate. Other frequent isolates include *Klebsiella*, *Enterobacter* and *Proteus* species, *Enterococcus* species (usually associated with instrumentation and catheterisation), and *Pseudomonas aeruginosa* (associated with structural abnormality or permanent urethral catheterisation). *S. aureus* rarely causes infection, and is associated with renal abnormality or as a secondary infection to bacteraemia, surgery or catheterisation. It is frequently seen as a contaminant due to perineal carriage. Other coagulase negative Staphylococci may cause complicated infections in patients of both sexes with structural or functional abnormalities of the urinary tract, prostatic calculi or predisposing underlying disease.

Types of urine specimen and collection

Midstream urine (MSU) and clean-catch urine

MSU and clean catch urines are the most commonly collected specimens and are recommended for routine use. Cleaning the area before sampling makes little difference to contamination rates[58-60].

Suprapubic aspirate (SPA)

Suprapubic aspirate (SPA) is seen as the ‘gold standard’ but is usually reserved for clarification of equivocal results from voided urine in infants and small children. Before SPA is attempted it is preferable to use ultrasound guidance to determine the presence of urine in the bladder[24].

Catheter urine (CSU)

‘In and out’, or intermittent self-catheterisation, samples are occasionally collected to ensure that they are contamination free.

Bag and pad urine

Bag urine is commonly collected from infants and young children, although it should be discouraged as pads are a more comfortable and easier method of collection[61,62]. Artificially elevated leucocyte counts may be seen as a result of vaginal reflux of urine, recent circumcision or confusion with round epithelial cells found in urine from neonates. Negative cultures provide useful diagnostic information, but significant growth should be confirmed with SPA.

Other specimens

Other specimens obtained during or as a result of surgery include those from ileal conduit, cystoscopy, nephrostomy and urostomy, prostatic massage/secretions. Specimens may also be taken after bladder washout.

Laboratory investigation of UTI

Laboratory investigation of UTI normally involves microscopy (or an alternative method of measuring cellular components) and quantitative culture (or an alternative non-culture method such as a semi-automated urine analyser) with the use of chemical screening methods in certain instances[63].

The three main methods for the detection of UTIs involve culture, non-culture semi-automated systems (eg particle counting, electrical impedance, colorimetric filtration, photometry, bioluminescence, radiometry) and chemical (eg leucocyte esterase, nitrite, protein, and blood detection):

Except in a few patient groups, interpretations of culture results are made with regard to clinical presentation, the presence or absence of pyuria (which are associated with infection) and squamous epithelial cells (SECs) (which indicate contamination).

A reference guide for the diagnosis of UTI is available for use by clinicians[64]. Clinical evaluation of the patient helps the interpretation of laboratory results and assists in the diagnosis of UTI.

Adequate internal control measures are critical, especially when chemical tests are deployed away from the laboratory near to the patient and where culture is not performed on the basis of negative results.

Microscopy

Microscopy is used to identify the presence of white blood cells (WBCs), RBCs, casts, SECs, bacteria and other cellular components in the urine. Semi-quantitative methods using a microtitre tray with an inverted microscope or a disposable counting chamber are recommended for routine use. This SMI contains a table of multiplicative factors based on the varying volumes of urine dispensed, the diameter of well and the field of vision diameter (refer to Appendix 1 and 2)[65].

Microscopy need not be performed on all urine samples where screening for asymptomatic bacteriuria is required (eg antenatal clinic screening) and may be omitted for such indications if in compliance with local protocols. Automated screening systems offer flexible, cost effective alternatives to microscopy[63]. Microscopy (or an alternative) is recommended for all symptomatic patient groups, to assist in the interpretation of culture results and the diagnosis of UTI.

Microscopy of uncentrifuged, unstained urine has been used as a method of screening for bacteriuria without the need for culture, but is unreliable to detect counts $<10^7$ colony forming units per litre (cfu/L), ie $<10^4$ colony forming units per millilitre (cfu/mL). The sensitivity increases if the specimen is centrifuged and/or stained[66].

In a carefully taken specimen, significant pyuria correlates well with bacteriuria and symptoms in most patients to suggest a diagnosis of UTI. Significant pyuria is defined as the occurrence of 10^7 or more WBC/L (10^4 WBC/mL), although higher numbers of WBC are often found in healthy asymptomatic women[67]. A level of $>10^8$ WBC/L ($>10^5$ WBC/mL) has been suggested as being more appropriate in discriminating infection.

RBCs - Laboratories should consult with local urologists regarding the reporting of RBCs in urine.

Casts – Casts are cylindrical protein mouldings formed in the renal tubules and often giving clues to renal pathology. Recognition of casts is important in helping to establish the existence of renal disease, but is less useful in the differentiation of renal disorders.

Large numbers of hyaline casts are associated with renal disease, but may also be found in patients with fever or following strenuous exercise. Cellular and densely granular casts indicate pyelonephritis or glomerulonephritis. RBC casts usually indicate glomerular bleeding and are excreted in large numbers in the acute phase of

post-streptococcal nephritis or rapidly progressive nephritis. Less commonly, epithelial cell and fatty casts accompany acute tubular necrosis and nephrotic syndrome.

Crystals – These may be asymptomatic or associated with the formation of urinary tract calculi. Some crystals such as cystine are rarely seen and may indicate an underlying metabolic disease.

Squamous epithelial cells – SECs are a useful indicator of the degree of contamination from the perineal region.

Non-culture methods

Semi-automated methods

Urine analyser systems are expensive and vary in their performance. They are intended to identify red and white blood cells, bacteria, yeasts, epithelial cells, mucus, sperm, crystals and casts (depending on the technology).

Urine analysers may be used to screen for 'negatives' to allow earlier reporting and to facilitate cost-effective processing[68,69].

If urine analysers are used as a screening procedure to reduce the number of urine samples set up for culture, then there is a need for a robust validation and the key performance parameter is sensitivity. The cut-off values of the bacterial and WBC counts used to screen out urines can usually be set by individual users using the Sysmex UF-100 demonstrated that they could achieve a high sensitivity (98%) only at the price of reduced specificity (25%) which meant that only 22% of their urines would not be cultured[70].

Each laboratory should set cut off values to achieve clinically relevant sensitivity and predictive values appropriate for the key local populations (children, pregnant women and patients who are immunocompromised) and under take appropriate validation and verification.

It may be prudent, regardless of the screening results, to culture all urines from certain patients such as those from children, pregnant women and patients who are immunocompromised. The rationale for this recommendation is that there are more severe consequences in missing infection in children and the potential absence of WBCs in urine from asymptomatic pregnant women or patients who are immunocompromised.

Currently available technologies include the following:

- flow cytometry: this works by measuring electrical impedance (for volume), light scatter (for size) and use of fluorescent dyes (for nuclear and cytoplasmic staining). The particles are characterised using these measurements, and the results are displayed as scattergrams. Sensitivity and specificity results can vary depending on the parameters and cut-offs employed. Cut-off criteria are chosen for an analyser to balance the levels of sensitivity and specificity required according to a local assessment of clinical need[71-73].
- particle recognition system: the urine specimen passes through the analyser and a camera captures up to 500 frames per specimen. Each image is classified by size, shape, contrast and texture features. This technology has been shown to be more reliable for identifying cellular components, but less suitable for detection of bacteriuria. For cases that appear to be borderline manual microscopy counts are still needed[74-76].

- microscopic urine sediment analysis: the autoanalyzer will homogenize the specimen and transfer it to a single use cuvette (volume aspirated: 2.0 ml, volume examined: 2.2 µl) which are centrifuge for few seconds. Afterwards, whole-field high definition images are obtained (15 per sample) and the software (Auto Image Evaluation Module AIEM) performs a morphological analysis of the particles, allowing them to be counted and classified[77].

Chemical screening tests

Non-culture chemical screening tests may be used for screening negative urines according to selected criteria[78-81]. Most chemical tests are available commercially as dipsticks and are quick and easy to use. Reading colour changes in dipstick strips using colorimetric measurement is preferred, as results are more reliable and reproducible, and free from observer error, particularly if an automated reading system is used[82,83]. Boric acid, and some antimicrobial agents such as nitrofurantoin, and gentamicin adversely affect the leucocyte esterase test.

Chemical tests for the presence of blood may be more sensitive than microscopy as a result of the detection of haemoglobin released by haemolysis. The absence of all four infection associated markers (blood, leucocyte esterase, nitrate and protein) had a greater than 98% negative predictive value and a sensitivity and specificity of 98.3% and 19.2% respectively according to one study[80].

Culture methods

There are several culture methods for the quantification of bacteria in urine. The easiest and most commonly used are the calibrated loop technique, the sterile filter paper strip and multipoint technology[84-86]. Of these, multipoint methodology using CLED or chromogenic media, are considered to be the most versatile and efficient for large numbers of specimens. Other methods include use of dipslides, pour plates and roll tubes. These methods are not recommended for routine use in this SMI but may be useful in specific circumstances and in accordance with local protocols.

Multipoint inoculation of CLED agar alone may contribute to the under reporting of mixed cultures that are more readily identified using chromogenic agar or a range of identification and susceptibility media. The culture of urine by multipoint methods may be automated, or performed manually using either microtitre trays containing agar or by using 9mm agar plates. Microtitre trays may be read manually or with an automated system where the resulting data are transferred to the laboratory information management system for reporting. Microtitre trays examined manually require background light and some form of magnification to facilitate the recognition of mixed cultures and small colonies.

Chromogenic media contain various substrates which permit presumptive identification of several common species through a change in either colony pigmentation or colour of agar. They perform satisfactorily compared to CLED and have the advantage that mixed cultures are easier to detect. However, chromogenic media from different manufacturers can vary in specificity, and are relatively expensive[84,87].

The use of agar plates (rather than microtitre trays) may lead to false negative reporting if antimicrobial substances present in some urines diffuse to neighbouring inocula. When this occurs, repeat culture of the affected inocula is required.

Interpretation of culture

Studies conducted in the 1950s remain the basis for interpreting urine culture results showing that bacterial counts of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) are indicative of an infection and counts below this usually indicate contamination[88]. The most common organism implicated in UTI in this group is *E. coli*[56].

In specific patient groups, counts between 10^8 cfu/L (10^5 cfu/mL) and 10^5 cfu/L (10^2 cfu/mL) may be significant[24,30,31]. A pure isolate with counts between 10^7 and 10^8 cfu/L (10^4-10^5 cfu/mL) should be evaluated based on clinical information or confirmed by repeat culture. Overall the confirmation of a UTI requires the demonstration of significant bacteriuria by quantitative culture (defined according to patient group or specimen type). Routine culture methods may not be sensitive enough to detect low bacteria levels (eg $\leq 10^7$ cfu/L / $\leq 10^4$ cfu/mL) and increased sensitivity will be achieved by increasing the inoculum size (see section 4.5.2).

Increased inoculum sizes are also required for persistently symptomatic patients without bacteriuria if the patient has recurrent “sterile pyuria”, or for specimens where lower counts are to be expected, such as SPAs or other surgically obtained urine.

Other urine investigations

Screening for antimicrobial substances

This may be useful to detect false negative cultures where the inoculum contains an antimicrobial agent which diffuses into the agar and inhibits bacterial growth. Where microtitre trays are used for multipoint culture the highest concentration of antimicrobial is localised to the small area of medium in the microtitre tray. Where agar plates are used for multipoint culture (rather than microtitre trays), both the primary and neighbouring inocula, may be affected as a result of the diffusion through the medium.

A seeded plate is inoculated after other plates and the absence of growth after incubation indicates the presence of an antimicrobial substance. The procedure is simple if multipoint replicating devices are used (see section 4.5.2) and may reduce further testing of the specimen (eg for fastidious organisms)[89].

Detection of urinary antigen for Legionella

Urinary antigen (UrAg) detection is a convenient and cost effective method of diagnosing Legionnaires’ disease[90]. Antigen becomes detectable soon after onset of symptoms and the test may remain positive for several weeks, even after other tests have become negative[91,92]. The majority of UrAg-positive cases have been found to be a result of infection from *L. pneumophila* serogroup 1[93]. Equivocal EIA results should be examined by a second person and repeated for serogroup 1[94].

Note: TheUrAG test may not be appropriate in cases of nosocomial or atypical pneumonia.

Where practical, respiratory samples should be obtained from all patients with positive Legionella urinary antigen tests, and these, (and culture isolates if available), should be sent to the reference laboratory for strain typing.

A sample should be retained at -20°C in the event that re-testing may be required because of legal action (take care to ensure preservation of the chain of evidence)[95].

Screening for *Salmonella* Typhi and *Salmonella* Paratyphi

S. Typhi and *S.* Paratyphi are present in urine in the early stages of typhoid and paratyphoid fever. Screening urines may be received from suspected cases and/or their contacts for selective enrichment and culture.

Diagnosis of *Schistosoma haematobium* infection

May be undertaken on urine taken at a specific time coinciding with maximum egg excretion, or on the terminal portion of voided urine. Haematuria is the most common presentation of *S. haematobium* infection. Chronic infection can lead to bladder cancer (see B 31 – Investigation of specimens other than blood for parasites).

Screening for *Chlamydia trachomatis and Neisseria gonorrhoea*

May be undertaken on urine specimens from patients with sterile pyuria or as part of investigation for infection with sexually transmitted disease.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[96,97]

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.”

Transport of urine specimens

Rapid transport, culture, or measures to preserve the sample aid reliable laboratory diagnosis. Delays and storage at room temperature allow organisms to multiply, which generate results that do not reflect the true clinical situation. Where delays in processing are unavoidable, refrigeration at 4°C is recommended or the use of a boric acid preservative may be beneficial[98-103].

Boric acid preservative at a concentration of 1–2% holds the bacterial population steady for 48–96 hours, and other cellular components remain intact[98,99]. Toxicity to certain organisms has been reported[100]. The toxic effect is delayed and often reflects underfilling of the container[101,104].

Boric acid increases the maximum permissible time for transport to the laboratory to up to 96hr[105].

It should be noted that boric acid may be inhibitory to some organisms and may inhibit tests for leucocyte esterase[99-101].

Note: It is essential to follow the manufacturer's instructions on sample volume in boric acid containers[104].

SI unit nomenclature

Although SI units have been adopted in other SMIs, they have been left as optional for urines. Most current literature still refer to the old nomenclature when defining 'significant bacteriuria'. The following is a list of metric units and their SI equivalents.

$\geq 10^5$ cfu/mL equivalent to $\geq 10^8$ cfu/L

$< 10^5$ cfu/mL equivalent to $< 10^8$ cfu/L

10^4 cfu/mL equivalent to 10^7 cfu/L

$< 10^4$ cfu/mL equivalent to $< 10^7$ cfu/L

10^3 cfu/mL equivalent to 10^6 cfu/L

$< 10^3$ cfu/mL equivalent to $< 10^6$ cfu/L

10^2 cfu/mL equivalent to 10^5 cfu/L

Validation and verification

Robust validation of the cut offs is required for your local area and should be carried out.

If urine analysers are used as a screening procedure to reduce the number of urine samples set up for culture, then there is a need for a robust validation and the key performance parameter is sensitivity. The cut-off values of the bacterial and WBC counts used to screen out urines can usually be set by individual users using the Sysmex UF-100 demonstrated that they could achieve a high sensitivity (98%) only at the price of reduced specificity (25%) which meant that only 22% of their urines would not be cultured[70].

Each laboratory should set cut off values to achieve clinically relevant sensitivity and predictive values appropriate for the key local populations (children, pregnant women and patients who are immunocompromised) and under take appropriate validation and verification.

Carry over contamination

Carry-over can be a problem with some automated urine analysers and the potential for this problem should be assessed during the validation and verification of these instruments[106,107]. There are a number of ways of addressing carry-over concerns such as increasing the number of rinses, but this reduces the throughput; furthermore with one specific analyser even increasing the number of the rinses did not prevent carry-over from specimens with very high bacterial load (10^7/mL)[108]. Other strategies

to reduce the carry-over, or its impact, include taking sample aliquots for culture before submitting the specimens for microscopy or culturing all urines before the microscopy is done, weekly disinfection of the probes with methanol, and regular carry-over tests using boric acid tubes[106,107].

1 Safety considerations[96,97,109-123]

1.1 Specimen collection, transport and storage[96,97,109-112]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[96,97,109-123]

Containment Level 2 unless infection with a Hazard Group 3 organism, for example *Mycobacterium* species, or *Salmonella* Typhi or *Salmonella* Paratyphi A, B and C is suspected.

Where Hazard Group 3 *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

Diagnostic work with clinical material that could possibly contain Hazard Group 3 organisms (*Salmonella* Typhi and *Salmonella* Paratyphi A, B & C,) does not normally require full Containment Level 3 containment[115] (paragraph 175).

If these Hazard Group 3 organisms are suspected, work should take place at a higher containment level, but full Containment Level 3 may not be required (paragraphs 179-183)[115].

If the work to be carried out requires the growth or manipulation of a Hazard Group 3 enteric biological agent then this has to be carried out under full Containment Level 3 conditions[115] (paragraph 175).

Note: *S*. Typhi and *S.* Paratyphi A, B and C cause severe, sometimes fatal, disease; laboratory acquired infections have also been reported. *S.* Typhi vaccination is available. Guidance is given in Immunisation against Infectious Disease published by Public Health England.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[115].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Bag urine, pad urine, catheter urine, prostate massage/secretions, clean catch urine, suprapubic aspirate, cystoscopy urine, ureteric urine, ileal conduit urine, urostomy urine, mid-stream urine, nephrostomy urine

Urine samples are not suitable for the isolation of leptospires due to the presence of other contaminating bacteria and the poor viability of leptospires in urine.

2.2 Optimal time and method of collection[124]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[124].

Mid-stream urine (MSU)

MSU is the recommended routine collection method.

The first part of voided urine is discarded and, without interrupting the flow, approximately 10mL is collected into a CE marked leak proof container. The remaining urine is discarded. If boric acid preservative is used, the container is filled up to the mark in a similar manner and the contents mixed well.

Clean-catch urine

A reasonable alternative to MSU.

Periurethral cleaning is recommended. The whole specimen is collected and then an aliquot sent for examination in a CE marked leak proof container.

Suprapubic aspirate (SPA)[24]

Urine is obtained aseptically, directly from the bladder by aspiration with a needle and syringe. The use of this invasive procedure is usually reserved for clarification of equivocal results from voided urine (eg in infants and small children). Ultrasound guidance should be used to show presence of urine in the bladder before carrying out SPA.

Catheter urine (CSU)

The sample may be obtained either from a transient (‘in and out’) catheterisation or from an indwelling catheter. In the latter case, the specimen is obtained aseptically from a sample port in the catheter tubing or by aseptic aspiration of the tubing. The specimen should not be obtained from the collection bag.

Bag urine

Used commonly for infants and young children. The sterile bags are taped over the freshly cleaned and dried genitalia, and the collected urine is transferred to a CE marked leak proof container. There are frequent problems of contamination with this method of collection.

Pad urine[125]

An alternative to collecting bag urine from infants and young children. After washing the nappy area thoroughly, a pad is placed inside the nappy. As soon as the pad is wet with urine (but no faecal soiling), push the tip of a syringe into the pad and draw urine into the syringe. Transfer specimen to a CE marked leak proof container. If difficulty is experienced in withdrawing urine, the wet fibres may be inserted into the syringe barrel and the urine squeezed directly into the container with the syringe plunger.

Ileal conduit – urostomy urine

Urine is obtained via a catheter passed aseptically into the stomal opening after removal of the external appliance. Results from this type of specimen may be difficult to interpret.

Cystoscopy urine

Urine is obtained directly from the bladder using a cystoscope.

Ureteric urine

Urine samples are obtained from one or both ureters during cystoscopy via ureteric catheters inserted from the bladder.

Urine samples may also be sent following nephrostomy, other surgical procedures, or bladder washout.

Meares and Stamey localisation culture method for diagnosis of prostatitis[23]

The following specimens are collected:

- The initial 5–8mL voided urine (urethral urine)
- MSU (bladder urine)
- Expressed prostatic secretions following prostatic massage
- The first 2–3mL voided urine following prostatic massage

Urine for *S.* Typhi and *S.* Paratyphi cultures

Any urine samples from suspected cases or contacts of cases.

Early morning urine

Ideally three entire, first voided, early morning urine specimens are needed for culture for *M. tuberculosis* (see B 40 - Investigation of specimens for *Mycobacterium* species).

Urine for *S. haematobium* detection

Total urine sample passed into CE marked leak proof container without boric acid preservative is required. Alternatively, a 24hr collection of terminal urine may be examined (see B 31 - Investigation of specimens other than blood for parasites).

Urine for parasites

For investigation of parasites see B 31 - Investigation of specimens other than blood for parasites.

2.3 Adequate quantity and appropriate number of specimens[124]

A minimum volume of 1mL for specimens in plain CE marked leak proof container for bacterial pathogens.

Fill to the line marked on containers with boric acid preservative according to manufacturers' instructions.

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

3 Specimen transport and storage[96,97]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed within 4hr if possible, unless boric acid preservative is used[102,103].

If processing is delayed for up to 48hr, refrigeration is essential[98]. Alternatively, the specimen may be collected in a CE marked leak proof container with boric acid preservative[99-101,126].

This increases the maximum permissible time for transport to the laboratory to up to 96hr[105] .

It should be noted that boric acid may be inhibitory to some organisms and may inhibit tests for leucocyte esterase[99-101,127].

Note: It is essential to follow the manufacturer's instructions on sample volume in boric acid containers[104].

4 Specimen processing/procedure[96,97]

4.1 Test selection

Divide specimen on receipt for appropriate procedures such as investigation for viruses (boric acid samples are unsuitable for viruses) and *C. trachomatis* depending on clinical details.

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy or alternative screening methods

4.4.1 Standard

Microtitre tray with an inverted microscope

Mix the urine gently, to avoid foaming.

Using a pipette and disposable tips, dispense known volume (~60μL, see 'Note 2' below) of mixed urine to a numbered well in a flat-bottomed microtitre tray. Make sure that the specimen covers the whole bottom surface area (the use of a template will facilitate matching the specimen and well number).

Allow to settle for a minimum of 5min, but preferably 10–15min, before reading with an inverted microscope.

Scan several fields in each well to check for even distribution of cells and urine.

Count the numbers, or estimate the range, of WBCs and RBCs per representative field and convert to numbers (or range) per litre.

Enumerate and record SECs.

Enumerate and record casts, if present, and state type.

Record if bacteria, yeasts, *Trichomonas vaginalis,* or significant crystals such as cystine are present.

UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England

All procedures for enumeration of cells should be carried out according to local protocols.

Note 1: This SMI contains a table of multiplicative factors to correct for variability in microtitre tray well size based on varying volumes of urine dispensed, diameter of well and field of vision diameter (refer to Appendix 1)[65]. The number of WBCs counted should be multiplied by the multiplicative factor to take into account all the variables. If the well size, volume of urine dispensed, diameter of well or field of vision diameter are altered, then the multiplicative factor needs to be re-calculated.

Note 2: If the microtitre tray is also to be used for culture by multipoint inoculation, it should be stored at 4°C until culture is performed (unless all specimens in the tray are preserved with boric acid when refrigeration is not necessary).

Note 3: Microscopy should not be performed on screening specimens sent exclusively for the isolation of *S.* Typhi and *S.* Paratyphi for safety reasons.

4.4.2 Alternative methods

See introduction for a discussion on non-culture methods.

Screening by biochemical test strips may be performed in place of microscopy; however, these methods do not detect casts or abnormal cells such as dysmorphic cells.

Automated systems such as those using urine analysers must be validated and used in accordance with manufacturers' instructions.

4.4.3 Supplementary

Microscopy for:

- **dysmorphic RBCs**[11,12] – Laboratories should consult with local urologists regarding the reporting of dysmorphic RBCs in urine. Fresh specimens (<30min old) are essential
- ***Mycobacterium* species** – (see B 40 - Investigation of specimens for *Mycobacterium* species)
- **parasites** – (see B 31 - Investigation of specimens other than blood for parasites)

4.5 Culture and investigation

4.5.1 Pre-treatment

Standard

N/A

Supplementary

Mycobacterium species (see B 40 - Investigation of specimens for *Mycobacterium* species) and for parasites (see B 31 - Investigation of specimens other than blood for parasites).

4.5.2 Specimen processing

Choice of culture method is made locally.

Specimens with 'negative' microscopy may be given a screening culture only, whereas those with 'positive' microscopy may include direct susceptibility testing.

Calibrated loop/surface streak method

Mix the urine gently to avoid foaming.

Dip the end of a sterile calibrated loop (eg 1µL, 2µL or 10µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on the shank[86].

Use this to inoculate CLED or chromogenic agar plate and spread according to the number of specimens (see Q 5 - Inoculation of culture media for bacteriology). A maximum of four samples per 9cm plate is recommended for this method with a 1µL or 2µL loop, or two samples if using a 10µL loop.

If a 1µL loop is used, one colony equals 1000 cfu/mL (ie 1 x 10^6 cfu/L).

SPAs, other surgically obtained urine, and urine samples with expected significant bacteriuria as low as 10^5 cfu/L (increased inoculum sizes are required)

Inoculate 100µL (0.1mL) of specimen aseptically to a full CLED or chromogenic agar plate.

Spread inoculum over entire surface of plate with a sterile loop or a spreader. Do not use a sterile swab which will absorb much of the inoculum. To isolate individual colonies, spread inoculum with a sterile loop.

No. of cfu/L = No. of cfu on plate x 10^4.

This semi quantitative method is only sensitive for screening down to 10^6 cfu/L if a 5µL or 10µL loop is used (eg 5 or 10 colonies), or 10^7 cfu/L if a 1µL or 2µL loop is used (eg 10 or 20 colonies). (See table below).

Guidance on assessing colony counts (with the exception of filter paper strip method; see Introduction for the clinical interpretation results)

	No. cfu counted using inoculum of:				
Corresponding cfu/L (cfu/mL)	0.3µL	1µL	2µL	5µL	10µL
10^6 cfu/L	-	-	-	5	10
10^7 cfu/L	3	10	20	50	100
10^8 cfu/L	30	100	200	500	1000

Multipoint methods

Using 96 pin head microtitre trays

Prepare microtitre tray and perform microscopy (see Section 4.4).

Label the microtitre tray containing chromogenic or CLED agar medium using the same template as for microscopy (see above Section 4.3.2).

Sterilise the inoculating pins on the multipoint inoculator.

Inoculate the agar microtitre tray with urine (eg 0.3µL, 1µL, 2µL, depending on pin size) from the 60µL aliquots used for microscopy.

Note 1: The tray must be stored at 4°C until full culture is performed (unless all specimens in the tray are preserved with boric acid).

Note 2: To prevent the inoculated agar in the microtitre trays from drying out in the incubator overnight, place the microtitre trays either in a moist box or stack carefully with a lid on the top tray.

Note 3: This method is only sensitive for screening down to 10^7 cfu/L. A larger inoculum may be required in selected patient groups or specimens when greater sensitivity is needed.

Using agar plates

Multipoint inoculation of no more than 20 specimens per 9cm plate is recommended.

Prepare inoculum in sterile cupules, arranged according to the configuration of the inoculation head.

Label CLED/chromogenic agar plate to correspond to inoculation configuration.

Sterilise the inoculating pins on the multipoint inoculator.

Dip inoculating pins into inoculum.

Inoculate CLED/chromogenic agar plate.

Note: Detection of antimicrobial substances must be undertaken if a multipoint culture method is used with agar plates, rather than microtitre trays, as diffusion of antimicrobial substances from some urine samples may affect neighbouring inocula and give false negative results (see section below: 'Detection of antimicrobial substances'). Any sample thought to be affected in this way should be retested.

Filter paper method[85]

Dip the commercially prepared sterile filter paper strip in the urine up to the mark indicated.

Remove excess urine by touching the edge of the strip against the side of the specimen container. Allow the remaining urine to absorb into the strip before inoculating a CLED or chromogenic agar plate.

Bend the inoculated end of the strip and press flat against the agar for a few seconds.

Several specimens may be inoculated onto one CLED agar plate in this technique, although this is less effective than plating to chromogenic agar, as mixed cultures are easier to detect[84].

Note: This method is only sensitive for screening down to 10^7 cfu/L. A larger inoculum will be required in selected patient groups or for specimens where lower counts are expected.

Guidance on assessing colony count using the filter paper strip method

No. cfu counted*		**Corresponding cfu/L (cfu/mL)**
Gram negative bacilli	**Cocci**	
0–5	0–8	10^7 ($\leq 10^4$)
5–25	8–30	10^7–10^8 (10^4 – 10^5)

25	30	10^8 (10^5)

*Refer to individual manufacturer's instructions.

Automated methods

Semi-automated systems such as urine analysers must be validated and used in accordance with manufacturers' instructions.

4.5.3 Other screening methods

Enteric fever screen

Enteric salmonellae may be recovered from urine following pre-enrichment in mannitol selenite, which can be prepared by carefully adding an equal volume of urine to mannitol selenite broth (see section: Safety Considerations).

Detection of antimicrobial substances[89]

This method is performed most easily using multipoint systems, but inoculation of urine is possible with a sterile loop or pipette and disposable tips.

Surface seed plates or microtitre tray containing a defined susceptibility testing agar with a broth culture or spore suspension of *Bacillus subtilis* (NCTC 10400) diluted to give a semi-confluent growth. *B. subtilis* is the preferred organism as it is susceptible to a wider range of antimicrobials than either *E. coli* or *S. aureus*.

Dry before use.

Inoculate plate or microtitre wells with urine as described earlier, ensuring that the seeded plate is inoculated last to prevent contamination of other media with *B. subtilis.*

4.5.4 Culture media, conditions and organisms[84]

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp. °C	Atmos.	Time		
UTI Screening in pregnancy for asymptomatic bacteriuria by culture	CLED agar or Chromogenic agar	35–37	Air	16–24hr	≥16hr	Enterobacteriaceae Enterococci Lancefield Group B streptococci Pseudomonads *S. saprophyticus* Other coagulase-negative staphylococci *S. aureus*
Enteric fever screen*	Mannitol selenite broth subcultured to: XLD	35–37 35–37	Air Air	16–24hr 16–24hr	N/A ≥16hr	*S.* Typhi *S.* Paratyphi
For these situations, add the following:						
Clinical details/ conditions	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
		Temp. °C	**Atmos.**	**Time**		
Urine of patients in Intensive Care, Special Care Baby Units, Burns Units and any from a Transplant Unit or if yeast have been seen in microscopy	Sabouraud agar	35–37	Air	40–48hr	≥40hr	Fungi
Multipoint culture using agar plates	Susceptibility testing agar seeded with *B.subtilis* (NCTC 10400)	35–37	Air	16–24hr	≥16hr	Antimicrobial substances
Optional media		**Incubation**			**Cultures read**	**Target organism(s)**
		Temp. °C	**Atmos.**	**Time**		
If sterile pyuria and no antimicrobials detected	Fastidious anaerobe agar	35–37	anaerobic	40–48hr	≥40hr	Anaerobes Streptococci
	Chocolate agar	35–37	5-10% CO_2	40–	≥40hr	Fastidious organisms

				48hr		
Susceptibility testing agar seeded with *B.subtilis* (NCTC 10400) (optional for all except multipoint agar plates)		35–37	Air	16–24hr	≥16hr	Antimicrobial substances
Other organisms for consideration – *C. trachomatis,* MRSA, *Mycobacterium* species, parasites and viruses (see relevant SMI). * These samples are rarely received by the laboratory.						

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Note: All work on *S.* Typhi and *S.* Paratyphi must be performed in a microbiological safety cabinet under Containment Level 3 conditions.

Anaerobes	"anaerobes" level ID 14 - Identification of anaerobic cocci ID 8 - Identification of *Clostridium* species ID 25 - Identification of anaerobic gram negative rods
β-haemolytic streptococci	Lancefield group level
Enterobacteriaceae (except *Salmonella* species)	"coliform" level
Enterococci	genus level
Pseudomonads	"pseudomonads" level
S. saprophyticus	species level
Other coagulase negative staphylococci	"coagulase negative" level
S. aureus	species level
S. Typhi/Paratyphi	species level
Yeasts	"yeasts" level
Mycobacterium	B 40 - Investigation of specimens for *Mycobacterium* species
Parasites	B 31 - Investigation of specimens other than blood for parasites
Fungi (in urines from patients species level in Intensive Care, Special Care Baby Units, Burns Units and any from Transplant Units)	species level

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC), EUCAST and/or CSLI guidelines or manufacturer's validation for proprietary methods.

This SMI recommends selective and restrictive reporting of susceptibilities to antimicrobials. Any deviation must be subject to consultation that should include local antimicrobial stewardship groups.

4.7.1 Antimicrobial Susceptibility Testing and Reporting Table

It is recommended that the antimicrobials in bold in the table below are reported. Those antimicrobials not in bold may be reported based on local decisions.

For more information on Detection of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases) refer to B 60.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
Enterobacteriaceae	**Ampicillin (or Amoxicillin)** Cefpodoxime[1] **Nitrofurantoin**[2] **Trimethoprim**	Amikacin Cefalexin Cefotaxime (or Ceftriaxone) Ceftazidime Ciprofloxacin (or Norfloxacin) Co-amoxiclav[3] Ertapenem Fosfomycin **Gentamicin** Mecillinam Meropenem (or Imipenem) Piperacillin/Tazobactam Temocillin Aztreonam	1. Cefpodoxime resistant organisms should be tested for the presence of ESBLs and screened for reduced susceptibility to carbapenems. 2. Nitrofurantoin for uncomplicated UTI only 3. Co-amoxiclav resistant organisms should be tested at a local level for sensitivity to an indicator carbapenem.
P. aeruginosa and *Acinetobacter spp.*	Ceftazidime (for *P.aeruginosa only)* **Ciprofloxacin** **Gentamicin** Meropenem (or Imipenem) **Piperacillin/Tazobactam**[4]	Amikacin Colistin	4. Tazobactam should not be rested or reported for *Acinetobacter spp.*

S. saprophyticus	**Cefoxitin**[5] **(or Oxacillin)** **Nitrofurantoin (uncomplicated UTI only)** **Trimethoprim**	Ciprofloxacin (or Norfloxacin) Gentamicin Penicillin Vancomycin	5. Report as Flucloxacillin
S. aureus and other coagulase negative Staphylococci	**Cefoxitin**[5] **(or Oxacillin)** **gentamicin** **Tetracycline**[6] **Trimethoprim** **Nitrofurantoin**	Clindamycin Daptomycin Fusidic acid Linezolid Mupirocin **Penicillin** Rifampicin Teicoplanin Vancomycin	5. Report as Flucloxacillin 6. Supress report in children and pregnant women
Group B Beta-Haemolytic Streptococci	**Nitrofurantoin**[2] **Penicillin** **Trimethoprim**	Clindimycin	2. Nitrofurantoin for uncomplicated UTI only may be useful in interpretation
Enterococcus spp.	**Ampicillin (or Amoxicillin)** **Nitrofurantoin**[2]	Teicoplanin Vancomycin	2. Nitrofurantoin for uncomplicated UTI only

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

5.1.1 Microscopy

Report on the actual numbers, or range of WBCs and RBCs per litre or per mL according to local protocol.

Report on the presence of bacteria, epithelial cells, casts, yeasts and *T. vaginalis.*

Report on supplementary microscopy for dysmorphic RBCs, (see B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (see B 31 - Investigation of specimens other than blood for parasites).

5.1.2 Chemical and semi automated screening methods

Report the results obtained together with a quantitative interpretation if applicable.

The following comments may be added:

"Culture not indicated – bacterial count below significant threshold".

"If symptoms persist or recur please submit a further sample indicating culture required".

5.1.3 Microscopy or chemical screening reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.1.4 Urine for antigen testing

Positives

Legionella pneumophila urine antigen positive

Comment – Provisional positive for *Legionella pneumophila* serogroup 1 antigen in urine. Please send a respiratory sample for Legionella culture. Specimen has been referred to the Reference laboratory for confirmatory testing.

Negatives

Legionella pneumophila serogroup 1 urine antigen not detected

5.2 Culture

Report bacterial growth in either metric or SI units, according to local protocol (see section: Technical Information/Limitations at end of Introduction).

Including comments where appropriate (refer to Appendix 3) or

Report no significant growth or

Report absence of growth.

Report presence of antimicrobial substances, if detected.

Report results of supplementary investigations.

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Supplementary investigations: *Mycobacterium* species (see B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (see B 31 - Investigation of specimens other than blood for parasites).

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

Refer to table 4.7.1. The table includes guidance on some of the of agents that should be tested on the bacterial isolates listed. The table also includes additional agents that can be considered for inclusion in test panels in specific clinical scenarios.

Any deviation from the guidance should be subject to local consultation and risk assessment.

Generally, all non-intrinsic resistant results should be reported as this is good practice and informs the user.

6 Notification to PHE[128,129], or equivalent in the devolved administrations[130-133]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[130,131], Wales[132] and Northern Ireland[133].

Appendix 1: Multiplicative factors based on varying volumes of urine dispensed, diameter of well & field of vision (Fov) diameter[65]

Volume of urine dispensed (µL)	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80
Diameter of well (mm)	8	8	8	8	8	8	7	7	7	7	7	7	6	6	6	6	6	6
FOV diameter (mm)	1.00	0.90	0.80	0.70	0.60	0.50	1.00	0.90	0.80	0.70	0.60	0.50	1.00	0.90	0.80	0.70	0.60	0.50
Multiplicative Factor	0.8	1.0	1.3	1.6	2.2	3.2	0.6	0.8	1.0	1.3	1.7	2.5	0.5	0.6	0.7	0.9	1.3	1.8
Multiplicative Factor (rounded)	1	1	1	2	2	3	1	1	1	1	2	2	0.005	1	1	1	1	2
Volume of urine dispensed (µL)	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
Diameter of well (mm)	8	8	8	8	8	8	7	7	7	7	7	7	6	6	6	6	6	6
FOV diameter (mm)	1.00	0.90	0.80	0.70	0.60	0.50	1.00	0.90	0.80	0.70	0.60	0.50	1.00	0.90	0.80	0.70	0.60	0.50
Multiplicative Factor	0.9	1.1	1.4	1.9	2.5	3.7	0.7	0.9	1.1	1.4	1.9	2.8	0.5	0.6	0.8	1.0	1.4	2.1
Multiplicative Factor (rounded)	1	1	1	2	3	4	1	1	1	1	2	3	1	1	1	1	1	2
Volume of urine dispensed (µL)	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Diameter of well (mm)	8	8	8	8	8	8	7	7	7	7	7	7	6	6	6	6	6	6
FOV diameter (mm)	1.00	0.90	0.80	0.70	0.60	0.50	1.00	0.90	0.80	0.70	0.60	0.50	1.00	0.90	0.80	0.70	0.60	0.50
Multiplicative Factor	1.1	1.3	1.7	2.2	3.0	4.3	0.8	1.0	1.3	1.7	2.3	3.3	0.6	0.7	0.9	1.2	1.7	2.4
Multiplicative Factor (rounded)	1	1	2	2	3	4	1	1	1	2	2	3	1	1	1	1	2	2

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Appendix 2: Multiplicative factor equation[65]

Multiplicative Factor for Appendix 1

$$\text{Multiplicative factor} = \frac{1}{\text{Volume observed } (V_o)}$$

$$V_o = \pi(\text{FOVr})^2 \times \text{fd}$$

$$\text{Fluid depth (fd)} = \frac{\text{Volume dispensed}}{\pi(\text{Radius of the well})^2}$$

<u>Where:</u>

π– Known as Pi whose constant value is known as 3.14

FOVr - The radius of the Field of Vision (in , calculated by dividing the diameter of the FOV which is calculated as FOV number (marked on eyepiece) divided by the objective magnification or by direct measurement with a micrometre.

Appendix 3: Guidance for the interpretation of urine culture

Note: This table is intended for guidance only - supplementation with local reporting policies may be necessary.

Growth cfu/L	No. isolates		Specimen type	Clinical details/microscopy influencing report*	Laboratory interpretation	Susceptibility testing recommended	Comments to consider
$\geq 10^8$	1		Any	None	Probable UTI	Yes	If old specimen or no pyuria consider repeat to confirm Consider SPA or CCU if bag specimen
	2	Each org $\geq 10^8$ or $\geq 10^8$ and $\geq 10^7$	MSU, CCU, SCU, BAG	WBC present Symptomatic	Possible UTI – colonisation, faulty collection or transport	Yes	Consider repeat to confirm
			CSU, (IL)	Indwelling catheter Neurogenic bladder	Probable colonisation	No – Consider keeping plates ≤ 5d in case patient becomes septic	Consider discuss if patient systemically unwell and therapy required
	2	1 organism predominant at $\geq 10^8$ or 10^7	Any	None	Possible UTI – ?colonisation, faulty collection or transport	Yes, predominant organism	If old specimen or no pyuria consider repeat to confirm Consider SPA or CCU if bag specimen
	≥3	Mixed growth - none predominant	Any	None	Faulty collection or transport	No	Heavy mixed growth – probable contamination. Consider repeat if symptomatic
$10^7 – 10^8$	1		Any	WBC present Symptomatic	Possible UTI – patient evaluation necessary	Yes	Consider repeat to confirm
	2	1 predominant at $\geq 10^7$	Any	WBC present Symptomatic Children	Probable UTI with predominant species 2nd isolate probable contamination	Yes, predominant organism but suppress results	Consider repeat or SPA/CCU Sensitivities are available if required
		1 at $< 10^7$ or – 10^8 but not predominant		None	Probable contamination	No	Mixed growth – probable contamination
	≥3	1 organism predominant at $\geq 10^7$	Any	WBC present Symptomatic	Possible UTI with predominant species. Others probable contamination	No – keep plates ≤ 5d if catheter specimen in case patient becomes septic	Mixed growth – consider repeat if symptomatic
		Any combination	CSU	Indwelling catheter Neurogenic bladder	Colonisation	No – keep plates ≤ 5d in case patient becomes septic	Please discuss if therapy indicated

Appendix 3 (continued): Guidance for the interpretation of urine culture

Note: This table is intended for guidance only – supplementation with local reporting policies may be necessary.

Growth cfu/L	No. isolates		Specimen type	Clinical details/microscopy influencing report	Laboratory interpretation	Susceptibility testing recommended	Comments to consider
10^6 – 10^7	1		MSU, CCU, CSU, IL	Symptomatic female Prostatitis WBC present	Possible UTI – clinical evaluation necessary	Yes	Consider repeat to confirm
	2	Each org ≥10^6 - including possible pathogen, eg *E. coli* or *S. saprophyticus*	Any		Possible UTI – clinical evaluation necessary	Yes	Consider repeat to confirm
10^5 – 10^8	1		SPA, CYS, (SCU)	None	Probable UTI	Yes	
	2	Each ≥ 10^6		WBC present	Probable UTI – patient evaluation necessary	Yes	
	≥3	1 organism predominant at ≥ 10^6		WBC present	Probable UTI – patient evaluation necessary	Yes, predominant organism	Mixed growth: Consider repeat to confirm
No growth		ie: < 10^6 if 1 µL loop used < 10^5 if 10 µL loop used	Any	None or asymptomatic	No UTI		
				Symptomatic Marked/persistent pyuria	• Patient on antibiotics? • Consider *Chlamydia*, AFB etc • Fastidious organism? • Consider bacteriuria < 10^5 cfu/L		As appropriate

MSU – Midstream specimen; CCU – clean catch; SPA – Suprapubic aspirate; IL– Ileal conduit; CSU –- Catheter; SCU – Single, intermittent catheter (“in and out”); CYS – Cystoscopy

* - see Introduction for interpretation of culture results

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References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	**Quality of evidence**
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, eg case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 41 Investigation of urine

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Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 07/09/2015 – 05/10/2015

Version of document consulted on: B 41dj+ 07/09/2015 – 24/09/2015

B 41dl+ 25/09/2015 – 05/10/2015

Proposal for changes

Comment number	1		
Date received	08/09/2015	**Lab name**	University Hospitals of Leicester NHS Trust
Section	Page 10 of 46		
Comment			
Pyonephrosis should be Pyelonephritis.			
Evidence			
Pyonephrosis is disease of the kidney and Pyelonephritis is the inflammation (infection) of the kidney. Source: medical books, dictionaries of Medical Terminology, etymology of Greek words.			
Recommended action	**ACCEPT** The SMI has been updated.		

Comment number	2		
Date received	11/09/2015	**Lab name**	Salford Royal NHS Foundation Trust
Section	4.7.1 Antimicrobial Susceptibility Testing and Reporting Table		
Comment			
a. *S. saprophyticus* row - Trimethoprim: Add penicillin? b. *S. aureus* and other Coagulase Negative Staphylococci row - Erythromycin: Not useful for urine. Replace with trimethoprim? c. *S. aureus* and other Coagulase Negative Staphylococci row - Penicillin: Unlikely to be useful? d. Group B Beta-Haemolytic Streptococci row - Clindamycin and Erythromycin: Not useful for urine.			
Recommended action	a. **ACCEPT** The SMI has been updated. b. **ACCEPT** The SMI has been updated.		

	c. **NONE** The antibiotic is there just for consideration. d. **PARTIAL ACCEPT** Clindamycin is now included which removes the need for Erythomycin.

Comment number	3		
Date received	23/09/2015	**Lab name**	Eumedica
Section	4.7 Antimicrobial susceptibility testing		
Comment			
I would like to comment on chapter 4.7. antimicrobial susceptibility testing and more particularly on table 4.7.1. I have acknowledged that due to a clerical error the version of the table presented in this document is incorrect but, while awaiting for the final version, I would like to emphasize the absolute need to add temocillin in this table for testing against Enterobacteriaceae. There are different reasons for such a request. First of all, temocillin is a narrow spectrum antibiotic with directed activity against the Enterobacteriaceae, the main organisms found in urinary tract infections. Secondly, temocillin is stable to most beta-lactamases including ESBL and AmpC enzymes which are frequently found in Enterobacteriaceae and against which very few antibiotics remain active. In this context, temocillin is now considered as carbapenem-sparing agent which is supported by in vitro, in vivo and clinical data. Finally, temocillin is mainly excreted by the urines with around 80% of the dose found in the urines in 24h. This pharmacokinetic parameter also support the use of temocillin for treating such infection. The United Kingdom is one of the few countries which has the opportunity to use this drug and it is unfortunate that it is not use more in clinical practice. All guidelines, including ESPAUR document, start smart then focus, etc... support the use of narrow spectrum antibiotics such as temocillin. To implement such guidelines, it is important that those antibiotics are tested at the same time as broad spectrum agent (carbapenem). Without this, how people will be able to spare the use of carbapenems?			
Recommended action	**ACCEPT** The SMI has been updated to include Temocillin.		

Comment number	4		
Date received	25/09/2015	**Lab name**	Royal Free London NHS Foundation Trust
Section	4.7		
Comment			
Enterobacteriaceae should be tested against Temocillin and Aztreonam. These drugs			

offer invaluable therapeutic options given that: a. PHE advise against use of ciprofloxacin and cephalosporins because of their association with *C. difficile*. b. Aminoglycosides are often unsuitable, particularly in renal impairment (AKI). c. Piperacillin/tazobactam is best reserved for infections where anti-pseudomonal activity is required. d. Carbapenems are best spared wherever possible given rising resistance rates. e. When an IV option is required, fosfomycin is not ideal. f. Co-amoxiclav resistance rates are around 30%.	
Evidence	
Balakrishnan I, Awad-El-Kariem FM, Aali A, Kumari P, Mulla R, Tan B, Brudney D, Ladenheim D, Ghazy A, Khan I, Virgincar N, Iyer S, Carryn S and Van De Velde S. Temocillin use in England: clinical and microbiological efficacies in infections caused by extended-spectrum and/or derepressed AmpC β-lactamase producing Enterobacteriaceae. J Antimicrob Chemother 2011;66:2628-2631. Livermore DM, Tulkens PM. Temocillin revived. J Antimicrob Chemother 2009; 63: 243-5. Kennedy H et al. Reduction in broad-spectrum Gram-negative agents by diverse prescribing of aztreonam within NHS Tayside J. Antimicrob. Chemother. (2015) 70 (8): 2421-2423.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	**ACCEPT** The SMI has been updated to include Temocillin and Aztreonam.

Comment number	5		
Date received	26/09/2015	**Lab name**	AMRHAI Ref Unit
Section	4.7.1		
Comment			
a. Given that many labs use disc testing, why are fewer than 6 antibiotics listed in the primary panels? Surely it makes sense to recommend full sets of 6 drugs even if reporting should then be limited to a subset of them. b. Why is amp / amox recommended in primary set but not co-amoxiclav? Many Enterobacteriaceae have acquired or intrinsic R to the unprotected agents. Consider need to test +/- clav in primary set.			

c. Suggest the document recommends that a carbapenem (MEM) should be included on primary panel so that we are consistent with the developing carbapenemase SMI (B 60?); all Gnegs to be tested for reduced carbapenem susceptibility / or resistance. d. Temocillin may be considered, has urinary breakpoint, and yet does not feature even as an unreported agent in the recommended testing panel. Consider adding to secondary panel.	
Evidence	
See UK NEQAS returns - top methods used by participants. See SMI B 60 (in development). See BSAC susceptibility testing website.	
Recommended action	a. **NONE** The aim of the table is not to recommend specific testing panels but to suggest which antibiotics should be considered in testing. b. **ACCEPT** A cross reference to B 60 – Screening and Detection of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases) has been added. c. **ACCEPT** A cross reference to B 60 – Screening and Detection of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases) has been added. d. **ACCEPT** The SMI has been updated to include Temocillin.

Comment number	6		
Date received	28/09/2015	**Lab name**	Imperial College Healthcare NHS Trust
Section	4.7.1 - Page 31		
Comment			
I am surprised there is no mention of Temocillin. As you are aware, Temocillin does provide a very good Gram negative cover against ESBL and AmpC producing organisms, sparing carbapenems. I would definitely add Temocillin to the list of supplementary testing. Please do not hesitate to contact me should you require any further information.			
Evidence			
J. Antimicrob. Chemother. (2010) 65 (suppl 3): iii25-iii33. doi: 10.1093/jac/dkq298.			
Financial barriers			

Not aware of any.	
Health benefits	
Not aware of any.	
Recommended action	**ACCEPT** The SMI has been updated to include Temocillin.

Comment number	7		
Date received	30/09/2015	**Lab name**	Lewisham and Greenwich
Section	4.7		
Comment			
a. Agents to be included within primary test panel: Should include Pivmecillinam. b. Agents to be considered for supplementary testing: Should include Temocillin and Aztreonam.			
Evidence			
a. Emerging clinical role of pivmecillinam in the treatment of urinary tract infection in the context of multidrug-resistant bacteria JAC 2013. b. Clostridium difficile infection: risk with broad-spectrum antibiotics NICE 2015.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	a. **NONE** The document contains Mecillinam. b. **ACCEPT** The SMI has been updated to include Temocillin and Aztreonam.		

Comment number	8		
Date received	01/10/2015	**Lab name**	PHE
Section	Antibiotic		
Comment			
Temocillin missing.			

Evidence	
Key antimicrobial agent for UTI.	
Health benefits	
I'm not sure I understand this question in the context of urine infection.	
Recommended action	**ACCEPT** The SMI has been updated to include Temocillin and Aztreonam.

Comment number	9		
Date received	01/10/2015	**Lab name**	Not stated
Section	4.7.1 Antimicrobial Susceptibility Testing and Reporting Table		
Comment			
Although there are many useful antibiotics listed as agents to be tested, Temocillin and Aztreonam are missing from this list. As a Trust, we use Temocillin as a Carbapenem-sparing agent and also in place of Tazocin (as Temocillin is less C.diffogenic) whenever possible. It is also part of our empirical antibiotic regimen for urosepsis. Because we use Temocillin very regularly, it is included in the 2nd line antibiotic susceptibility testing panel. Adding Temocillin and Aztreonam to the list of antibiotics to be considered may help promoting antimicrobial stewardship.			
Evidence			
Livermore DM1, Tulkens PM. Temocillin revived. J Antimicrob Chemother. 2009 Feb;63(2):243-5. doi: 10.1093/jac/dkn511. Epub 2008 Dec 18.			
Recommended action	**ACCEPT** The SMI has been updated to include Temocillin and Aztreonam.		

Comment number	10		
Date received	05/10/2015	**Lab name**	Ashford and St Peter's NHS foundation Trust
Section	Antimicrobial susceptibility testing page 31-32		
Comment			
a. Under Enterobacteriaceae: I am surprised that Temocillin is not considered for supplementary testing as its very stable against ESBLs and AMPC and testing is happening in many UK centres already where it's used for that purpose and other indications in order to preserve Carbapenems and Piperacillin/tazobacatm and reduce Antimicrobial resistance.			

b. Also I am surprised that Aztreonam is not there also. c. Mecillinam should perhaps be there as it is an oral option. d. Co-amoxiclav too as amoxicillin and ampicillin have higher level of resistance. e. Nothing is under CPE which is a new emerging Global threat.
Evidence
Many UK labs including our centre test for Temocillin, Aztreonam and Mecillinam. The evidence is in the following published references: Livermore et.al; Temocillin revived : Journal of Antimicrobial Chemotherapy (2009) 63, 243-245 Balakrishnan et.al; Temocillin use in England: clinical and microbiological efficacies in infections caused by extended-spectrum and/or derepressed AmpCb-lactamase-producing Enterobacteriaceae :J Antimicrob Chemother 2011; 66: 2628-2631 Habayeb et.al; Amoxicillin plus temocillin as an alternative empiric therapy for the treatment of severe hospital-acquired pneumonia: results from a retrospective audit: Eur J Clin Microbiol Infect Dis. 2015 Aug;34(8):1693-9.
Financial barriers
No.
Health benefits
No.

Recommended action	a. **ACCEPT** The SMI has been updated to include Temocillin. b. **ACCEPT** The SMI has been updated to include Aztreonam. c. **NONE** Mecillinam is present. d. **ACCEPT** A cross reference to B 60 – Screening and Detection of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases) has been added. e. **ACCEPT** A cross reference to B 60 – Screening and Detection of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases) has been added.

Comment number	11		
Date received	05/10/2015	**Lab name**	GHNHSFT/RCPATH
Section	4.7		

Comment	
In the era of carbapenem sparing and CDI target driven healthcare it amazes that Temocillin has not been included in the panel of agents to be considered for supplementary testing for urine.	
Evidence	
CMO Report Balakrishnan et al. Temocillin use in England: clinical and microbiological efficacies in infections caused by extended-spectrum and/or derepressed AmpC β-lactamase-producing Enterobacteriaceae. JAC 2011 Nov;66(11):2628-31 Woodford N et al. In vitro activity of temocillin against multidrug-resistant clinical isolates of Escherichia coli, Klebsiella spp. and Enterobacter spp., and evaluation of high-level temocillin resistance as a diagnostic marker for OXA-48 carbapenemase. JAC Sept 29 Paterson DL and Doi Y. Activity of Temocillin against KPC-Producing Klebsiella pneumoniae and Escherichia coli AAC 2009 53(6) Habayeb H et al. Amoxicillin plus temocillin as an alternative empiric therapy for the treatment of severe hospital-acquired pneumonia: results from a retrospective audit. Eur J Clin Microbiol Infect Dis 2015 Aug 34(8) - evidence of less all cause diarrhoea and less CDI Guidance for Carbapenem-resistant enteriobacteriaceae (CRE) 2012. CDC guidance to reduce multi-drug resistant gram negative bacteria (mdrgnb) infections - Scottish Antimicrobial Prescribing Group and SMC guidance October 2013 Pallett A & Hand K. Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria JAC 2010 65 Suppl3	
Financial barriers	
Yes related to carbapenem prescribing restrictions and targets.	
Health benefits	
No.	
Recommended action	**ACCEPT** The SMI has been updated to include Temocillin and Aztreonam.

Targeted questions

Do you agree with the concept of including antimicrobial susceptibility testing and reporting tables in SMIs?		
Date received	**Lab name**	**Comment**
23/09/2015	Eumedica	Yes.
25/09/2015	Royal Free London NHS Foundation Trust	Yes - useful guide for prioritisation.
26/09/2015	AMRHAI Ref Unit	Yes - one of the objectives for PHE towards affecting the UK AMR Strategy is to achieve better standardisation of

		antibiotic panels tested. Strong recommendations made through the SMI documents will help this.
28/09/2015	Imperial College Healthcare NHS Trust	Yes - yes, it will help to standardize testing across the country.
30/09/2015	Lewisham and Greenwich	Yes - agree in principle but needs to allow room for changes based on local sensitivity pattern and prescribing guidelines.
01/10/2015	PHE	No - I feel they should default to the expert guidance already provided by EUCAST and BSAC as they are updated more frequently than SMI documents. National and European guidance is responsive to change and encourages surveillance; this guidance is limited and almost discourages the testing of a wider range of agents.
01/10/2015	Not stated	Yes.
05/10/2015	Ashford and St Peter's NHS foundation Trust	Yes - I think it’s highly important document to produce and terrific piece of work but it will be great if you incorporate our comments please.
05/10/2015	GHNHSFT/RCPATH	Yes - easy to find and access.

Do you agree with the content of the antimicrobial susceptibility testing and reporting table in this SMI?

Date received	Lab name	Comment
23/09/2015	Eumedica	No.
25/09/2015	Royal Free London NHS Foundation Trust	No - see comment 4.
26/09/2015	AMRHAI Ref Unit	No - see comment 5.
28/09/2015	Imperial College Healthcare NHS Trust	No – see comment 6.
30/09/2015	Lewisham and Greenwich	Yes - also standard reporting comments.

01/10/2015	PHE	No - as described above I don't feel that antimicrobial testing guidance should be included. Were the tables of agents to be tested and reported based on national surveillance information? HPA gathered UTI data from all the Vitek machines at regional labs for 2 years, did this show any evidence to justify the range of agents tested. Augmentin R is not a specific enough marker for CPE and would result in far too much supplementary testing. Where is the evidence for this comment? All national and internal guidance suggests screening for CPE using Mer or Ert; indicating that this guidance is already out of step with current practice.
05/10/2015	Ashford and St Peter's NHS foundation Trust	Yes - in general yes with the exception of NOT including temocillin susceptibility testing and CPE.
05/10/2015	GHNHSFT/RCPATH	No – comment 11.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	02/09/2015	**Lab name**	Microbiology at Hairmyres Hospital

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Investigation of bone and soft tissue associated with osteomyelitis

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Bacteriology | B 42 | Issue no: 2 | Issue date: 14.12.15 | Page: 1 of 28

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Acknowledgments

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Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 5

SCOPE OF DOCUMENT 7

INTRODUCTION 7

TECHNICAL INFORMATION/LIMITATIONS 13

1 SAFETY CONSIDERATIONS 15

2 SPECIMEN COLLECTION 15

3 SPECIMEN TRANSPORT AND STORAGE 16

4 SPECIMEN PROCESSING/PROCEDURE 16

5 REPORTING PROCEDURE 21

6 NOTIFICATION TO PHE, OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 22

APPENDIX 1: INVESTIGATION OF BONE AND SOFT TISSUE ASSOCIATED WITH OSTEOMYELITIS BY CULTURE 23

REFERENCES 24

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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	4/14.12.15
Issue no. discarded.	1.3
Insert issue no.	2
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope.	Updated for clarity.
Introduction.	Re-organised and streamlined. Updated to include Waldvogel and Cierry-Mader classifications, spondylodiscitis and rapid techniques.
Technical information/limitations.	Updated to include limitations of UK SMIs.
Specimen transport and storage.	Section 3.1. IDSA Guidelines for transport included: Transport at room temperature, and should be processed immediately, and within a maximum of 2hr.
Specimen processing/procedure.	Section 4.3.1. Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised. Addition of information regarding molecular testing.
Culture and investigation.	Culture media, conditions and organisms updated. Direct FAA plate removed. Addition of molecular testing.
Reporting.	Reporting text updated. Addition of molecular testing.
Appendix.	Flowchart updated to reflect culture media table.

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UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

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While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Intra-operative samples of bone, bone biopsies, soft tissue, aspirates

This SMI describes the processing and microbiological investigation of bone and soft tissue associated with osteomyelitis and includes information regarding molecular techniques.

For biopsies and aspirates sent for the investigation of prosthetic joint infections refer to B 44 – Investigation of orthopaedic implant associated infections.

This SMI should be used in conjunction with other SMIs.

Introduction

Osteomyelitis is a progressive infection which results in inflammation of the bone and causes bone destruction, necrosis and deformation[1]. In children the growing ends of long bones are the most common site of infection whereas in adults it is the spine[2,3]. Risk factors for adult (haematogenous) osteomyelitis include sickle cell disease, immune deficiencies and intravenous drug use[4]. Organisms most often isolated from bone and soft tissue samples include[5]:

- *Staphylococcus aureus*
- coagulase negative staphylococci
- *Enterococcus* species

Gram negative bacteria and fungi may also be isolated. Gram negative bacilli when isolated are of major clinical importance due to their antimicrobial resistance patterns[6].

Classification

Two classification systems of osteomyelitis are currently in use; the Waldvogel classification and the Cierny-Mader classification[7,8].

The Waldvogel classification is based on the pathogenesis of disease. Categories are defined by the duration of illness (acute/chronic), the source of infection (eg contiguous focus originating from local infected tissue) and vascular insufficiency (eg diabetic foot infection)[7,9].

There are several limitations to the Waldvogel classification; it does not include infection caused by direct inoculation into the bone, for example caused by trauma, and due to the fact that it is based on pathogeneses of disease, it does not lend itself to use in clinical practice[9].

The Cierny-Mader classification is a clinical 'staged' classification. Initially cases are categorised into one of four stages of osteomyelitis; stage 1 (medullary), stage 2 (superficial), stage 3 (localised) or stage 4 (diffuse)[9,10]. The patient is then categorised as either A, B or C. Patients in category A do not have systemic or local compromising factors, those in category B are affected by systemic and/or local compromising factors and those patients in category C are severely compromised, and treatment is considered worse than the disease[9].

There are also several limitations to the Cierny-Mader method of classification. Placing patients into category C is subjective and categorisation may differ between clinicians. Also, the classification does not take the duration of the disease into consideration[9].

For the purpose of this SMI the structure of the introduction has been arranged based on the Waldvogel classification because of its etiological basis.

Acute contiguous-focus osteomyelitis

In contiguous-focus osteomyelitis, the organisms may be inoculated at the time of trauma or during intra-operative or peri-operative procedures. Alternatively they may extend from an adjacent soft tissue focus of infection. Common predisposing factors include surgical reduction and fixation of fractures, prosthetic devices, open fractures and chronic soft tissue infections (see B 14 – Investigation of abscesses and deep seated wound infections). In general the microbiology of contiguous osteomyelitis is more complex than that of haematogenous osteomyelitis and is commonly polymicrobial.

Contiguous-focus osteomyelitis without vascular insufficiency

Puncture wounds of the foot through footwear such as training shoes are particularly associated with osteomyelitis due to *Pseudomonas aeruginosa*[11-13]. Osteomyelitis following human bites and tooth socket infections affecting the mandible are often caused by strict anaerobes for example *Actinomyces* species; in children anaerobic bone and joint infections are rare[14-16].

Contiguous-focus osteomyelitis with vascular insufficiency

Most patients with contiguous-focus osteomyelitis associated with vascular insufficiency have diabetes mellitus. The bones and joints of the feet are most often affected[9].

Diabetic foot infections are responsible for many hospital admissions and a significant number can end up with limb amputation and consequent disability[17-19]. Neuropathy and vasculopathy (impaired blood supply) are complications of diabetes. The former means that protective sensation is lost, allowing skin injury to occur without it being perceived. In addition it can ultimately lead to fragmentation, destruction and dislocations of the bones of the foot (Charcot neuro-osteoarthropathy). Foot deformity in diabetics due to motor neuropathy is also a further strong risk factor for developing ulcers and infection. The basic principles in the treatment of diabetic foot infection are education and prevention with good glucose control, accommodative footwear, regular inspection and general compliance.

Once infection has occurred, abscesses may need to be drained, diagnostic biopsies may be required to guide antibiotics and diseased bone may need to be resected. Acute infections in patients who have not recently received antimicrobials are often monomicrobial (almost always with aerobic Gram positive cocci such as *S. aureus* and β-haemolytic streptococci), whereas chronic infections are often polymicrobial. Cultures of specimens obtained from patients with such mixed infections generally yield 3–5 isolates, including Gram positive and Gram negative aerobes and anaerobes. These may include enterococci, various Enterobacteriaceae, obligate anaerobes, *Pseudomonas aeruginosa* and other non-fermentative Gram negative rods. Hospitalisation, surgical procedures, and, especially, prolonged or broad spectrum antibiotic therapy may predispose patients to colonisation and/or infection

with antibiotic resistant organisms (eg meticillin resistant *Staphylococcus aureus* (MRSA) or vancomycin resistant enterococci (VRE)). The impaired host defences around necrotic soft tissue or bone may allow low-virulence colonizers, such as coagulase negative staphylococci and *Corynebacterium* species ("diphtheroids"), to assume a pathogenic role.

In the immunocompromised or diabetic host, *Nocardia* species should also be considered as a rare cause of osteomyelitis[20].

Acute haematogenous osteomyelitis[1,2,4,21]

Haematogenous osteomyelitis has been classically described in childhood, but can occur in any age group especially when there are risk factors such as a recent intravascular device, haemodialysis, intravenous drug usage or recurrent infections elsewhere (such as urinary tract infections)[4]. In adults the vertebrae are most often affected, however the long bones, pelvis or clavicle may also be affected[22].

In classical haematogenous osteomyelitis of childhood, the growing ends (metaphyses) of long bones are involved. The commonest organism is *S. aureus*; however β-haemolytic streptococci and HACEK organisms such as *Kingella* species are also important causes[21]. Organisms in the bloodstream gain access to bone by way of the nutrient artery. They pass through branches of this vessel to the small blind ended terminal vessels usually near the epiphyseal plate (growing end of the bone). This area is thought to have sluggish circulation, and bacteria can lodge here, starting the process of infection. Following this there is extension to other areas and the host inflammatory response is mobilised. Pus is created and expands under pressure thereby creating further impedance of the local circulation and death of bone.

In certain areas such as the hip, where the epiphyseal plate is situated within the joint capsule, early joint involvement by infection is common. Pus under pressure may strip the periosteum (outer lining of bone). New immature bone is formed as a response to periosteal stripping, and, in severe cases, the entire shaft may be encased in a sheath of new bone referred to as an involucrum. Where a major portion of the shaft has been deprived of blood supply, a resulting sequestrum (dead bone) lies within the involucrum. Openings in the bone may permit escape of pus from bone causing abscesses, systemic sepsis and in some cases death.

The bacterial species in haematogenous osteomyelitis are usually dependent on the age of the patient. In neonates, Group B streptococci, *S. aureus* and *Escherichia coli* cause infection[1]. Multiple sites of infection are common in neonates[23]. Between the ages of one and sixteen, *S. aureus*, and *Haemophilus influenzae* type b predominate (although the latter is rare after the age of five years and increasingly rare in children under five because of a successful vaccination campaign). *Streptococcus pneumoniae* is occasionally involved. In adult life, *S. aureus* is the commonest organism and, in the elderly, infection with aerobic Gram negative rods may occur. *Candida* species may be found when intravenous devices are in use[24]. In acute haematogenous osteomyelitis a single pathogenic organism is usually isolated but in many cases of chronic osteomyelitis, particularly when associated with wounds and ulcers the disease can be polymicrobial.

Salmonella species rarely cause osteomyelitis in patients who are immunocompetent; typically infections with *Salmonella* species (usually non-typhi serotypes) are associated with sickle cell anaemia (see below), other haemoglobinopathies or patients who are immunocompromised[25]. *Salmonella* osteomyelitis normally affects

the diaphysis of long bones (usually the femur or humerus) and the vertebrae[25]. Infection by indirect contamination from an animal host has been reported[26,27].

Spondylodiscitis[3]

The term spondylodiscitis refers to vertical osteomyelitis, discitis and spondylitis. These are manifestations of haematogenous osteomyelitis, which may result from the same pathological process and may occur at the same time[3,28]. Spondylodiscitis is responsible for 3-5% of all osteomyelitis cases, and it is the main cause of osteomyelitis in patients aged over 50 years[3].

In adults, organisms may enter the discs via the arteries causing inflammation; infection may then extend to the vertebral column[28,29]. Vertebral osteomyelitis may also result from trauma or complications during surgery. Risk factors include older age, a recent intravascular device, haemodialysis, diabetes and intravenous drug usage (a risk factor for Pseudomonas infection), infection and immunosupression[30]. Lumbar spine infections may originate from urinary tract infections, possibly by translocation of bacteria via a venous plexus (Batson's plexus) that links the bladder with the spine. Following the initial infection, pus may break out of the cortex anteriorly to form a paravertebral abscess or posteriorly to form an epidural abscess. In addition weakening of the bone may cause vertebral collapse. Organisms causing vertebral infections include *S. aureus,* streptococci and aerobic Gram negative rods (associated with urinary tract infections)[1].

In patients with risk factors, tuberculosis should always be considered; microbiological diagnosis (with or without histology) is required for a definitive diagnosis[31,32]. Although infection most often occurs in the spine, extrapulmonary tuberculosis can occur in any bone or joint. Diagnosis is mainly by biopsy for histology and microbiology. Cultures are often positive and are crucial for determining the presence of resistance to anti-tuberculosis agents. However the decision to treat is often made on clinical and histopathological grounds in the first instance[33,34]. Other mycobacteria, such as *Mycobacterium marinum, Mycobacterium avium-intracellulare, Mycobacterium fortuitum* and *Mycobacterium gordonae* have also been associated with bone infections particularly in patients who are immunocompromised.

In endemic areas *Brucella* species are a common cause of vertebral infection, therefore a travel history should always be sought. Other fastidious Gram negative rods eg the HACEK group (*Haemophilus* species*, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella* species (see ID 12 – Identification of *Haemophilus* species and the HACEK group of organisms) may be occasional causes of vertebral osteomyelitis[35].

Sickle cell disease[1,9]

Adult haematogenous osteomyelitis in adults is often associated with sickle cell disease. Symptoms may mimic those of marrow crisis; culture results should therefore be used for confirmation of clinical diagnosis. Organisms often isolated include *Salmonella* species, *S. aureus* and streptococci.

Haemodialysis patients[9]

As a result of the use of intravascular access devices in these patients, haematogenous infections can occur usually due to *S. aureus* or coagulase negative staphylococci. Gram negative infections are more common in haemodialysis patients than in the general population.

Intravenous drug users[9,36]

Septic arthritis and osteomyelitis of the long bones or vertebral discs are associated with haematogenous infection in intravenous drug users. Organisms often isolated include *S. aureus*, *P. aeruginosa* and *Candida* species.

Chronic osteomyelitis[1,9,22]

Patients typically present with chronic pain and drainage, and may have a history of previous osteomyelitis at the same site. Treatment of infection may be challenging as surrounding tissue and bone will be of poor quality; antibiotic treatment alone is rarely sufficient to arrest infection. Risk factors include open fractures, bacteraemia and ischemic ulcers associated with diabetes, sickle cell disease and malnutrition. Organisms often associated with chronic osteomyelitis include *S. aureus,* Gram negative bacilli and anaerobic bacteria.

Device related osteomyelitis

In chronic device related infections, organisms may be present in a biofilm that is associated with the device or diseased/necrotic bone. Refer to B 44 – Investigation of orthopaedic implant associated infections.

Haematogenous

Acute haematogenous osteomyelitis can lead to chronic osteomyelitis characterised by dead areas of bone and sinus tract[37,38]. This condition can fail to respond to treatment and persist for long periods[10]. Infections may recur many years after the first episode[39].

Brodie's abscess[40]

Brodie's abscess is an uncommon condition and is a chronic localised abscess of bone, most often in the distal part of the tibia. It is usually due to *S. aureus* and generally occurs in patients under 25 years of age. Surgery (surgical debridement) and culture-specific antibiotic therapy are usually effective in arresting infection.

Fungal osteomyelitis

Fungal osteomyelitis is rare; however, some fungi endemic to certain areas can be associated with osteomyelitis. This includes *Cryptococcus, Blastomyces* and *Sporothrix* species. In patients who are immunocompromised or those with multiple previous surgical procedures at that site, more common fungi such as *Candida* or *Aspergillus* species can also cause osteomyelitis[24,41]. A mycetoma is a chronic granulomatous infection of the skin, subcutaneous tissues and in its advanced stages, bone. It is most prevalent in tropical and sub-tropical regions of Africa, Asia and Central America. Infection usually follows traumatic inoculation of organisms into subcutaneous tissue from soil or vegetable sources[42]. Various genera have been implicated including *Madurella, Acremonium, Pseudoallescheria* and *Actinomadura* species. Mycetoma may also be caused by bacteria[43]. It is characterised by subcutaneous granulomata containing grains and can lead to infection of the bone. Black grains are indicative of fungal infection and the condition known as eumycetoma. Actinomycetoma is caused by bacteria, and grains are white, yellow or red[44]. Infection normally occurs through a puncture wound (normally of the foot); however infection can occur in the legs, hands or arms[43]. Deep surgical biopsy is required to obtain viable samples for microbiological culture[44].

Diagnosis[9,19,21,22]

The diagnosis of osteomyelitis usually requires a combination of a full clinical assessment, plain X-rays and further imaging (eg MRI scan, CT scan, ultra-sound), blood cultures (particularly in acute cases), bone and/or soft tissue biopsies and/or surgical sampling. For specific indications eg risk of Brucella infection, other tests such as serology may be required. When tuberculosis is suspected, a full clinical 'work up' including a chest X-ray is indicated.

Sample types

Radiologically obtained percutaneous bone biopsies

These may be taken in the radiology department where they can be guided by imaging such as ultrasound, fluoroscopy or CT. Usually a sample should also be sent to histology to confirm infection, provide pointers to unusual infections and/or exclude malignancy. It is not commonly possible to send more than one sample to microbiology, but when this is done, each should be processed separately. It is important that detailed clinical information is provided to ensure cultures are set up for appropriate organisms. This includes details such as the presence of a prosthetic device (where any organism eg a coagulase negative staphylococcus, may be the pathogen and also where prolonged cultures are required). It also includes any clinical suspicion or risk factors for tuberculosis, brucella, nocardia, atypical mycobacteria or fungi.

Intra-operative bone biopsies

These are taken in theatre either as primarily a diagnostic procedure, or as the first part of a larger debridement/resection procedure. Multiple (4-5) samples should be taken from separate site using separate sterile instruments for microbiological culture. Similar samples from similar sites should also be taken for histopathological examination. A risk-benefit assessment of antibiotic timing is required. Where infection is likely and/or a microbiological diagnosis is likely to significantly affect clinical outcome, prophylactic antibiotics can be withheld until immediately after sampling. The effect of a single dose of antibiotic on the sensitivity of microbiological culture is unknown. In addition to bone samples, deep soft tissue samples are usually taken at the same time. Sinus samples should be discouraged as colonising organisms cannot be differentiated from infecting organisms.

Samples from around devices[45]

Samples of bone and soft tissues may be taken from around a prosthetic device, eg a fracture fixation plate or nail. Samples associated with such devices should be processed with the same principles as those associated with prosthetic joint samples (B 44 – Investigation of orthopaedic implant associated infection).

Enrichment culture

Blood culture systems, where bottles are incubated for up to five days, have shown equivalent sensitivity to conventional enrichment broth for the culture of orthopaedic implant associated specimens (refer to B 44 – Investigation of orthopaedic implant associated infection)[46,47]. Similar studies have not yet been published regarding their use for bone and soft tissue specimens associated with osteomyelitis.

Management[9,19,21,22]

In acute presentations, surgery may be required to drain pus. In chronic osteomyelitis, areas of dead bone may need to be resected. Both need to be accompanied by specific antibiotic therapy depending on culture results. This is most often carried out intravenously, initially. In some cases, where the disease is too extensive to fully resect, the patient is too unfit for surgery, or a device is retained, long term oral antibiotics may be required. Organisms need to be tested against a wide variety of antibiotic options as patients commonly are intolerant of one or more antibiotics.

Rapid techniques[48-50]

Molecular methods[50-52]

Nucleic acid amplification techniques - NAAT (eg PCR) for the identification of bacteria, fungi, parasites and viruses from clinical samples have been shown to be highly specific and sensitive[50,51,53]. PCR targets conserved genes within the genome, and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short timeframe particularly if multiplex real-time PCR is used.

PCR has been shown to be more sensitive than conventional culture for the isolation of some fastidious organisms for example *Kingella kingae*, and PCR – hybridization after sonication has been shown to improve diagnosis of implant related infections[54,55]. There are however some issues with NAATs analysis. A lowered sensitivity may be observed due to the small volume of samples processed, in some cases there may be interference with human DNA originating from the tissue samples, and antibiotic susceptibility information is not available[56,57].

MALDI-TOF mass spectrometry[48,49]

Recent developments in identification of bacteria and yeast include the use of 16s ribosomal protein profiles obtained by matrix assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectrometry. Mass peaks achieved by the test strains are compared to those of known reference strains. It is possible for an organism to be identified from an isolate within a short time frame and it is increasingly being used in laboratories to provide a robust, rapid and effective identification system for bacterial and yeast isolates.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers[58,59]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical

Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”

1 Safety considerations[58-74]

1.1 Specimen collection, transport and storage[58-63]

Use aseptic technique.

Care should be taken to avoid accidental injury when using “sharps”.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[58-74]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[66].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Bone, bone biopsies, soft tissue, aspirates

2.2 Optimal time and method of collection[75]

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[75].

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[76-80].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Direct collection in theatres can be placed into a CE marked leak proof container with Ringer’s or saline solution and Ballotini beads (as an option) which is placed into sealed plastic bags. However, microbiology and histology specimen pots can be confused leading to difficulties in processing samples.

2.3 Adequate quantity and appropriate number of specimens[75]

In surgery for chronic osteomyelitis collection of multiple (4-5) intra-operative samples with separate instruments (usually sterile forceps and scalpel) is important. Duplicate samples must be taken for histology. Swabs are not recommended.

Minimum specimen size will depend on the number of investigations requested.

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

3 Specimen transport and storage[58,59]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[75]. To enable timely clinical management, samples should be processed urgently.

The Infectious Diseases Society of America (IDSA) guidelines recommend that specimens should be transported at room temperature, and should be processed immediately, and within a maximum of 2hr[75].

If processing is delayed, refrigeration is preferable to storage at ambient temperature.

If possible stop all antibiotics at least 2 weeks prior to sampling and consider not giving routine surgical prophylaxis until after sampling[81].

The volume of the specimen influences the transport time that is acceptable. Larger pieces of bone may maintain the viability of anaerobes for longer[82]. Samples should not however exceed the size of the CE marked leak proof containers available.

4 Specimen processing/procedure[58,59]

4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) or fungi depending on clinical details.

4.2 Appearance

N/A

4.3 Sample preparation

To enable timely clinical management, samples should be processed urgently. Non-repeatable samples should be prioritised.

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

Examine the specimen for the presence of any soft tissue. Remove soft tissue using a sterile scalpel or scissors and homogenise using, as appropriate, a sterile grinder (Griffith tube or unbreakable alternative), a sterile scalpel or (preferably) sterile scissors and Petri dish. The addition of a small volume (approximately 0.5mL) of sterile filtered water, saline or nutrient Ringer's will aid the homogenisation process.

Homogenisation must be performed in a racked shaker for 15 minutes in a Class 1 exhaust protective cabinet.

Note: Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised[83].

Optional

N/A

Supplementary

Fungi and *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species).

4.3.2 Specimen processing

Standard

Bone (percutaneous biopsy or intra-operative sample) or soft tissue associated with osteomyelitis

The objective should be to reduce manipulation to a minimum (for instance the number of times any container is opened), thereby minimising the risk of exposing the operative sample to potential contamination. For this reason centrifugation of the sample for concentration should not be performed, instead divide the whole sample in appropriate amounts for tests.

In units with high workloads of this specimen type, the provision to the operating theatre of CE marked leak proof containers in a sealed plastic bag with approximately 10 Ballotini beads and 5mL broth could be considered. In such circumstances, homogenisation can be carried out in the original container. It is not uncommon, however, for microbiology and histology specimen pots to be confused leading to difficulties in processing samples.

Alternatively, samples may be sent to the laboratory in a plain CE marked leak proof container in a sealed plastic bag. These samples require transfer, homogenisation and then further transfer to culture media, including liquid media. If this methodology is followed, particular care is necessary with asepsis when transferring, homogenising or processing the sample. Clean air provision is desirable. Homogenisation with Ballotini beads can be performed by adding the sample to a universal with approximately 10 Ballotini beads and 5mL of sterile saline (or Ringers solution) then shaking at 250 rpm for 10 minutes in a covered rack on an orbital shaker, or alternatively vortexing for 15 seconds (40 Hz). If molecular analysis is to be carried out then sterile molecular grade water and new universal containers should be used. In the case of molecular work the volume should not exceed 2mL.

Inoculate each agar plate and a slide for Gram staining with a drop of the suspension using a sterile pipette (see Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum using a sterile loop. Inoculate broth with the remainder of the suspension including any tissue fragments.

Optional

Specimens collected into appropriate CE marked leak proof containers should be used for microscopy and may be used for molecular techniques. Specimens for molecular testing should be processed according to manufacturer's instructions.

Supplementary

Fungi and *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species).

4.4 Microscopy

4.4.1 Supplementary

Gram stain (see TP 39 - Staining procedures)

Gram stains should be carried out on all pus samples and may be carried out on other sample types where clinically indicated. If sufficient specimen is received prepare as recommended in Section 4.5. Using a sterile pipette place one drop of specimen on to a clean microscope slide.

Spread this with a sterile loop to make a thin smear for Gram staining.

Note: Gram stain on tissue can be difficult to interpret and yield can be low.

4.5 Culture and investigation

Inoculate each agar plate using a sterile pipette (Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	**Atmos**	**Time**		
‡Osteomyelitis Brodie's abscess, Diabetic foot osteomyelitis, Discitis +For debridement of fracture fixation device refer to B 44 – Investigation of prosthetic joint infection.	Bone, Bone biopsy Soft tissue Aspirate	Blood agar and Chocolate agar	35 - 37	5 - 10% CO_2	40 - 48hr	Daily	Staphylococci Streptococci Enterobacteriaceae Pseudomonads HACEK group *Nocardia* species*
		Fastidious anaerobic broth, cooked meat broth or equivalent Subculture onto plates if evidence of growth, or at day 5 as below:	35 - 37	Air	5 d	N/A	Staphylococci Streptococci Enterobacteriacece Pseudomonads Anaerobes
Subculture plates	Bone, Bone biopsy Soft tissue Aspirate	FAA	35 - 37	Anaerobic	40 - 48hr	≥40hr	Anaerobes
		Chocolate agar	35 - 37	5 - 10% CO_2	40 - 48hr	Daily	Any

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	**Atmos**	**Time**		
Deep seated fungal infection#	Bone, Bone biopsy Soft tissue Aspirate	Sabouraud agar (slopes)	28 - 30	Air	14 d	Daily‡	Yeast Mould

Optional molecular techniques

Clinical details/ conditions	Specimen	Molecular technique	Instructions	Target organism(s)
All clinical conditions	Bone marrow	NAAT	Follow manufacturer's instructions	Any organism

Always consider other organisms such as *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species), fungi and actinomycetes. Routine processing for mycobacteria should be considered for all non-post operative spinal infections.

* If infection with *Nocardia* species is suspected, samples may require incubation for a further 3 days.

** Subcultures should be examined periodically (ideally daily) and subcultured if there is evidence suggestive of growth. Terminal subcultures should be performed at 5 days.

+Most surgical cases with intra-operative biopsies eg fracture fixation devices or chronic osteomyelitis requires multiple samples. If an indistinguishable organism is isolated in two or more samples then it is likely to be clinically significant.

‡ Extended incubation may be required (for up to 8 weeks) for certain species of fungi such as *Cryptococcus* species or *Histoplasma* species[84,85].

When investigating mycetoma, deep surgical biopsy is required to obtain viable samples for microbiological culture. Samples should be submitted in normal saline[44]. (Refer to B 17 – Investigation of tissues and biopsies).

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Actinomycetes	genus level ID 15 – Identification of anaerobic *Actinomyces* species
Anaerobes	genus level ID 14 - Identification of anaerobic cocci ID 8 - Identification of *Clostridium* species ID 25 - Identification of anaerobic gram negative rods ID 15 – Identification of anaerobic *Actinomyces* species
β-haemolytic streptococci	Lancefield group level
Other streptococci	species level
Enterococci	species level
Enterobacteriaceae	species level
Yeast and Moulds	species level
Haemophilus	species level
Pseudomonads	species level
S. aureus	species level
Staphylococci (not *S. aureus*)	genus level
Mycobacterium species	B 40 - Investigation of specimens for *Mycobacterium* species

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: Any organism considered to be a contaminant may not require identification to species level.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC), and/or EUCAST guidelines, or CSLI guidelines where applicable.

It is important to include a wide range of antibiotics particularly for those patients who may require prolonged oral treatment with biofilm active drugs (see Introduction). These antibiotics are not usually included in the common first line antimicrobials tested in most laboratories. For Gram positive organisms these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracyclines, quinolones, co-trimoxazole, fusidic acid, linezolid or quinupristin/dalfopristin.

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Standard

Gram stain

Report on WBCs and organisms detected.

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

Following results should be reported:

- clinically significant organisms isolated
- other growth
- absence of growth

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Also, report results of supplementary investigations.

Supplementary investigations: B 40 - Investigation of specimens for *Mycobacterium* species.

5.3 Molecular

Refer to manufacturer's instructions.

5.4 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[86,87], or equivalent in the devolved administrations[88-91]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

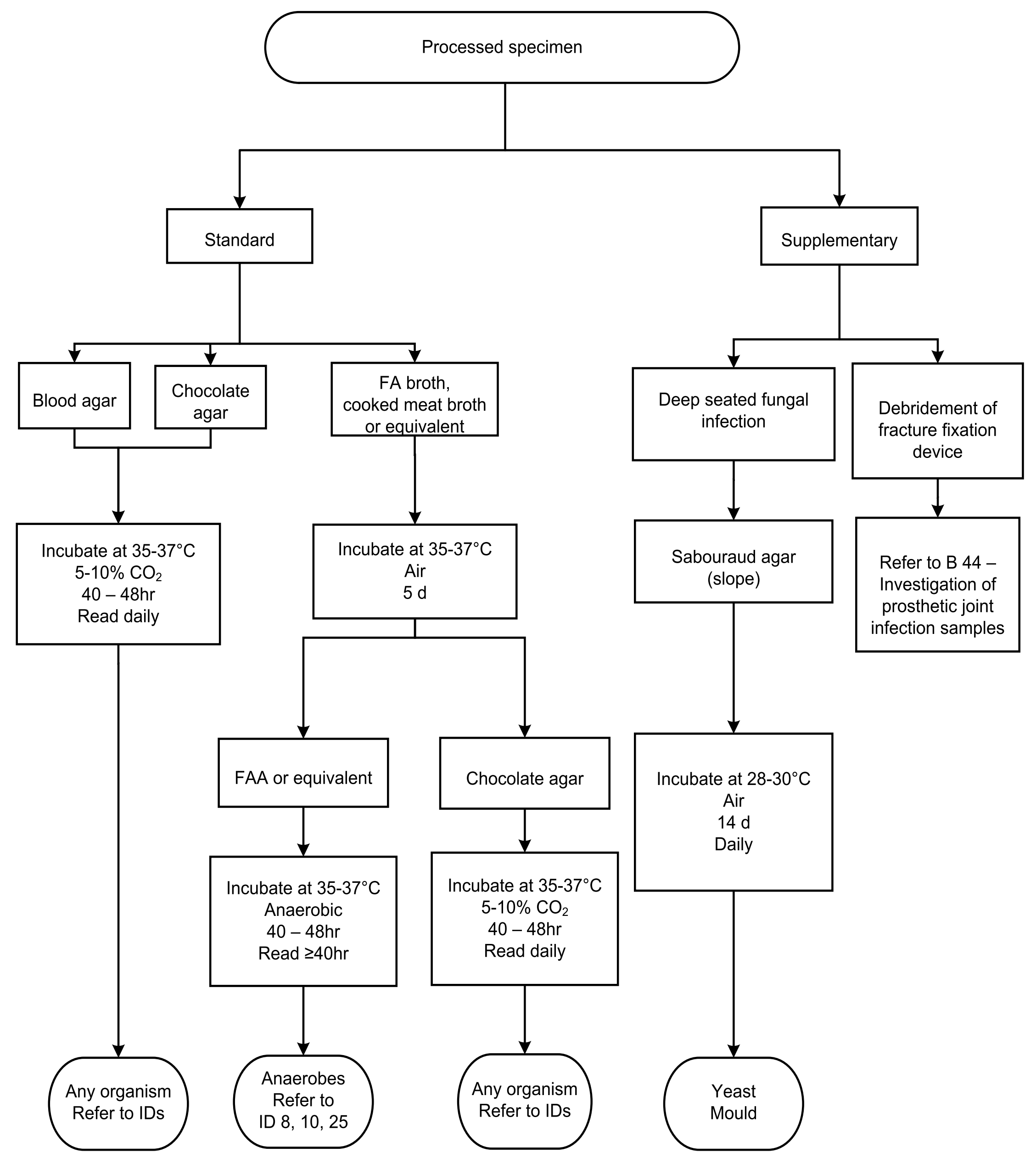
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[88,89], Wales[90] and Northern Ireland[91].

Appendix 1: Investigation of bone and soft tissue associated with osteomyelitis by culture



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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 42 Investigation of bone and soft tissue associated with osteomyelitis

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation: 09/08/2013 – 01/11/2013

Version of document consulted on – B 42dl+

Proposal for changes

Comment number	1		
Date received	29/10/2013	**Lab name**	Oxford
Section	Several		
Comment			
Osteomyelitis heading secondary to contiguous focus osteomyelitis is unnecessarily confusing. Would change to device related osteomyelitis. Otherwise no comments.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** Text updated.		

2nd Consultation: 15/09/2014 – 13/10/2014

Version of document consulted on – B 42dq+

Proposal for changes

Comment number	1		
Date received	22/09/2014	**Lab name**	Bone Infection Unit, Oxford
Section	Introduction		
Comment			
Introduction Misspelling of Cierny-Mader several times. A few minor edits needed in the wording to represent Waldvogel classification properly and to make the document read properly.			
Recommended action	**ACCEPT** Document updated.		

Comments received outside of consultations

Comment number	1		
Date received	01/07/2013	**Lab name**	MSTAG
Section	a. Introduction b. 2.5.3 c. 2.5.3. note d. 2.5.3 e. General note f. References g. Introduction		
Comment			
a. Could there be more detail on *Salmonella* and discitis? b. General comments - a lot of asterisks and notes. c. May require incubation for a further 3 days-when? d. Anaerobic plates-would not necessarily look at $AN0_2$ plates daily. e. Sabouraud incubation time 2-5d but examine at 40h and up to 8 weeks - inconsistent. f. Is it not recommended that tissue samples that are suspected to contain fungi are NOT homogenised-see B17? g. Waldvogel classification-has this now been superseded by Cierny classification?			
Recommended action	a. **PARTIAL ACCEPT** Text added to the introduction on *Salmonella* species. b. **NONE** Notes are required for clarification of points made within the table. This is the standard format. c. **ACCEPT** *'If infection with Nocardia species is suspected, samples may require incubation for a further 3 days.'* d. **ACCEPT** Table updated in line with other UK SMIs to state reading at ≥40hr. e. **ACCEPT** Following discussion at the working group meeting the incubation has been amended to 14d, read daily. A footnote regarding extended incubation has been added. f. **ACCEPT**		

	Text added to section 4.3.1. g. **PARTIAL ACCEPT** Both classifications are currently in use. Text has been added to the introduction regarding classification and the limitations of both. For the purpose of this document the etiological Waldvogel classification has been used.

Comment number	2		
Date received	02/08/2013	**Lab name**	BIA
Section	a. Section 1.2.2 b. Table 2.5.3: Mycetoma c. Section 3.3 d. Section 3.3 / 2.7 e. Section 2.6.1		
Comment			
a. Surely, can we endorse delays in the processing of samples up to 48hrs (merely stating that this is undesirable?). We may need to consider describing situations were delays are acceptable; in most it would constitute bad practice. b. You state that cultures need to be incubated for up to 5 days, but read for up to 8 weeks – looks like an inconsistency that needs to be ironed out. c. The bulk of section 3.3 refers to selection of antibiotics to be tested, and should therefore be moved to section 2.7. d. The following sentence is included in 2.7. Antibiotics can only be used if reported. Therefore, the sentence, 'Prudent use of antimicrobials according to local and national protocols is recommended.' should be in section 3.3. I really think that we need to consider advising that antibiotics should be reported selectively. This is a policy matter and would apply across numerous specimen types. e. Should we explicitly state that multiple organisms detected (particularly in contiguous focus cases) should be identified (and susceptibility performed) individually as default, ie not reported as 'mixed faecal flora' as sometimes happens?			
Evidence			
a. Clearly some form of support for this is needed. Aside from particular organisms that would be less likely to survive if stored, I suspect that it would be in the form of professional advice. I have no doubt that you have reviewed the relevant publications. If none is available, we'll need to consider whether an opinion on this can be given.			
Recommended action	a. **ACCEPT** This has been removed from the document.		

	b. **ACCEPT** Table updated. Extended incubation may be required (for up to 8 weeks) for certain species of fungi such as *Cryptococcus* species or *Histoplasma* species. Morris AJ, Byrne TC, Madden JF, Reller LB. Duration of incubation of fungal cultures. J Clin Microbiol 1996;34:1583-5. Bosshard PP. Incubation of fungal cultures: how long is long enough? Mycoses 2011;54:e539-e545 c. **ACCEPT** Text moved to Section 2.7. d. **ACCEPT** Currently this sentence is part of the template and is included in section 2.7. Text moved to section 3.3. Selective reporting is currently under discussion and will be assessed for this document at the next full review. e. **NONE** This was discussed and group felt that the minimum level of identification section of the document was appropriate in its current form.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	29/09/2014	**Lab name**	PHW

UK Standards for Microbiology Investigations

Investigation of orthopaedic implant associated infections

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, National Infection Service, PHE

Bacteriology | B 44 | Issue number: 2.1 | Issue date: 18 August 21 | Page: 1 of 34

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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see the Steering Committee page on GOV.UK).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

PHE publications gateway number: GOV-9410

UK Standards for Microbiology Investigations are produced in association with:

Contents

Acknowledgments 2

Amendment table 4

1. General information 7

2. Scientific information 7

3. Scope of document 7

4. Background 7

5. Safety considerations 9

6. Diagnostic tests and investigation 10

7. Antimicrobial susceptibility testing 24

8. Notification to PHE or equivalent in the devolved administrations 24

9. Referral to reference laboratories 25

Appendix: Investigation of orthopaedic implant associated infections by culture[a, b] 26

References 28

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number / date	5 / 18 August 2021
Issue number discarded	2
Insert issue number	2.1
Section(s) involved	Amendment
Whole document	Updated to new template
Appendix	Incubation of FA broth or equivalent corrected to 35-37 in air. Removed incubation at 5-10% CO2

Amendment number / date	4 / 23 February 2016
Issue number discarded	1.3
Insert issue number	2
Section(s) involved	Amendment
Whole document	Title changed to 'Investigation of orthopaedic implant associated infections'. References updated throughout.
Scope	Fixation devices added to types of specimen. Scope updated to reflect new title and use of molecular techniques.
Introduction	Re-structured and streamlined for clarity. Information regarding sample types, sampling, sample processing and molecular methods inserted and updated (previously in Technical Information/Limitations). Synovial white cell count cut offs for acute and chronic prosthetic joint infection included.

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<table>
<tr><td></td><td>Section on rapid techniques expanded to include MALDI-TOF MS.
Information on sonication included.
Gram staining in elective revision cases should not be considered for diagnosing infection.
Recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system method, extended to 14 days in some cases.</td></tr>
<tr><td>Technical information and limitations</td><td>Information included on sonication, small colony variants, contamination and effect of antibiotic use.</td></tr>
<tr><td>Safety considerations</td><td>Avoid sharps where possible. Needless syringes are available for use with blood culture bottles.
Ideally analysis should be carried out in a Class II cabinet.</td></tr>
<tr><td>Specimen collection</td><td>If possible stop antibiotics 2 weeks prior to surgery.
Swabs are discouraged.
In theatres multiple (3 to 5) samples should be taken.</td></tr>
<tr><td>Specimen transport and storage.</td><td>IDSA recommend samples are processed within 2 hrs.</td></tr>
<tr><td>Specimen processing or procedure</td><td>Ideally analysis should be carried out in a Class II cabinet.
Primary plates may not be required in some situations.
Enrichment broth/blood culture broth should be incubated for 5 days (extended to 14 days in certain situations).
Method for total white and differential leucocyte counts included.
Culture media, conditions and organisms table updated:
• Standard media
    o FAA, read at 3 and 5 day.
    o Inclusion of continuous monitoring blood culture system.</td></tr>
</table>

	◦ Broth incubation 5 days, extended to 14 days in certain situations. • Supplementary media ◦ Sabouraud agar, incubate for 14 days. • Addition of molecular techniques (NAAT). Identification – isolates should be kept for at least 2 weeks. Antimicrobial susceptibility testing updated to include extensive antibiograms.
Reporting procedure	Updated to include total white and differential cell counts. Culture reporting updated in line with new template. Molecular reporting added.
Appendix	Updated in line with section 4.5.3 Culture media, conditions and organisms.

*Reviews can be extended up to 5 years subject to resources available.

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1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

This UK SMI describes the microbiological investigation of orthopaedic implant associated infection samples and includes information on molecular techniques.

For information regarding bone and tissue samples associated with osteomyelitis refer to B 42 – Investigation of bone and soft tissue associated with osteomyelitis.

This UK SMI should be used in conjunction with other UK SMIs.

4. Background

Since the earliest hip replacements, pioneered in the UK by Sir John Charnley in the early 1960s, joint replacement (arthroplasty) has become a common procedure. It is done most commonly for osteoarthritis and inflammatory arthopathies such as rheumatoid arthritis. For hip fractures, a hemiarthroplasty is one of the surgical treatment options. Hip and knee replacements are more common than replacements of shoulder, elbow, ankle and interphalangeal joints (1). Bilateral replacements for osteoarthritis are common in weight bearing joints and multiple joint replacements are common in inflammatory arthritis. Revision surgery is done for joint failure (usually loosening or recurrent dislocation) and the majority are ‘aseptic’. Around 15% of revisions are due to ‘septic’ loosening (2).

4.1 Risk factors for infection

Infection rates are now much lower than when joint replacement was first introduced. There is however still a risk associated with each procedure. This is around 1 to 2% for elective hip and knee replacements and higher for emergency trauma operations, for example, hemiarthroplasties (3, 4). The risk of infection in a joint replacement is increased by patient factors, including; the early development of a surgical site infection not apparently involving the prosthesis, a National Healthcare Safety Network (NHSN) Score of 1 or 2, the presence of malignancy and previous joint arthroplasty(5 to 7). The NHSN score encompasses both surgical and patient factors such as the duration of surgery, degree of wound contamination and patient’s preoperative health (7). Other co-morbidities such as immunosuppression, diabetes, renal failure, heart or lung disease, smoking and obesity also increase the risk of infection after surgery, as does prolonged post-operative wound drainage and haematoma formation (5, 8, 9).

Surveillance of surgical site infections may be used as a means to prevent such infections which are associated with considerable morbidity and extended hospital stay (10, 11).

4.2 Pathogenesis and microbiology

Organisms may be introduced into the joint during primary implantation surgery, via a haematogenous (bloodstream) route or from post-operative contamination of the wound(12). These may cause acute or chronic infections. Fewer organisms are required to establish infection when there is a foreign body *in situ* than otherwise. The most common organism to cause acute infections is *Staphylococcus aureus* (meticillin sensitive or resistant) and in chronic infections either *S. aureus* or coagulase negative staphylococci. It is estimated that up to 30% of *S. aureus* bacteraemia may be associated with septic arthritis in those with pre-existing prosthetic joints (12). Many other organisms can be acquired by either direct inoculation or the haematogenous route including other skin flora, streptococci, coliforms, enterococci and rarely anaerobes, mycobacteria or fungi (3, 13, 14).

Once infection is established around a prosthetic joint, organisms can form a ‘biofilm’(15). Organisms secrete extracellular substances to produce a complex and sometimes highly organised glycocalyx structure within which they are embedded. In these microbial communities, which may be polymicrobial, some organisms are dividing slowly if at all, and others may even be in a state akin to dormancy. In the microbiological diagnosis of infection, this biofilm may have to be disrupted in order to culture organisms (16). The ‘persisters’ within the biofilm are very difficult to kill so that infection may not be eradicated without removal of the prosthesis. If it is to be retained, antibiotics with activity against organisms which form biofilms should be used, but standard antimicrobial sensitivities may not predict the required antimicrobial activity (17). In vitro models testing activity of antimicrobials against organisms within a biofilm are not at present feasible in routine laboratories (9).

4.3 Clinical presentation

Prosthetic joint infections can present acutely, with a hot, swollen painful joint. The patient is often febrile and can be clinically septic. Inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are usually raised(17). This presentation needs to be differentiated from acute inflammatory arthritides such as rheumatoid arthritis, gout, pseudogout and also from an acute haematoma (blood) in the joint. Alternatively, prosthetic joint infections can present chronically. The joint may simply be painful and stiff. There may be evidence for loosening of the prosthesis on X-ray. Inflammatory markers may be slightly raised, but this is not specific (17). These presentations are often difficult to differentiate from those of mechanical pain or aseptic loosening (18). The presence of a discharging sinus however, indicates the presence of a deep prosthetic joint infection.

4.4 Diagnosis

In the acute presentation of prosthetic joint infection, in addition to a full clinical assessment of the patient, blood cultures should be taken and a joint aspirate performed. An ultrasound may aid this and will clarify whether there is fluid in the joint itself. Synovial fluid may be visibly purulent or merely turbid. Plain X-rays are performed to look for early loosening, fracture or other pathology. Plain X-rays may show loosening, but do not differentiate septic from aseptic loosening. In the chronically infected prosthetic joint, the diagnosis is much more difficult. A past history of early post-operative wound infection increases the likelihood of deep infection. If

changes are rapidly progressive over time, infection is more likely. Nuclear radiology may have a role in diagnosis but scans can be non-specific or technically difficult to perform. Magnetic Resonance Imaging (MRI) and computerised tomography (CT) scans are rarely helpful. Inflammatory markers may only be slightly raised and are not specific or sensitive. Sinus cultures are not helpful as organisms cultured do not predict those causing deep infection (19). A joint aspirate for cell count, culture and histology, or periprosthetic joint biopsy for microbiology and histology (using ultrasound or other dynamic imaging), are the most specific tests for infection. As organisms may be in a 'sessile' biofilm form (rather than 'planktonic' and loose in the joint fluid) the sensitivity of a joint aspirate can be poor.

4.5 Interpretation of results

Defining organisms in separate samples as indistinguishable can be difficult. One or two differences in an extended antibiogram may not always indicate strains from different clonal origins. In addition, infection of prostheses with multiple strains can occur (2). It is important to perform sensitivity testing on all isolates from all samples as the extended antibiogram is a common and cheap way to identify strains as indistinguishable in multiple cultures and the presence of resistant strains will affect the outcome of therapy.

Organisms can be cultured from 60 to 70% of samples taken from prostheses deemed infected (2). As the organisms that cause chronic prosthetic joint infection are frequently the same as those that contaminate microbiological samples, interpretation of results is difficult when only 1 or 2 samples are taken. When 5 samples are taken, the false positive rate with 2 or 3 samples positive is less than 5% whereas false positive rates close to 30% are seen with a single positive sample (2). Growth of an indistinguishable organism from 2 or more samples is 71% sensitive and 97% specific. Recovery of an indistinguishable organism from 3 samples is 66% sensitive and 99.6% specific (2). Obtaining organisms from a single tissue sample therefore poses significant challenges in interpretation. Even with careful sampling and prolonged cultures, there is still a significant culture negative rate, even when histology is positive. This may be due to sampling error (the distribution of organisms can be patchy), very small numbers of organisms that do not thrive in laboratory culture conditions, an inability to disrupt organisms from the biofilm, unculturable organisms or false positive histology results. Immunofluorescent and molecular studies suggest that, in some cases, there may be organisms present even when conventional cultures are negative (2).

5. Safety considerations

Care should be taken to avoid accidental injury when using 'sharps'. The use of sharp objects should be avoided wherever possible. Sterile, needleless syringes and blood transfer devices are commercially available which may be used for the aseptic transfer of sample homogenate into blood culture bottles.

Use aseptic technique.

Compliance with postal, transport and storage regulations is essential.

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet (20).

Ideally, microbiological analysis should be carried out in a Class II cabinet, using aseptic technique, to reduce the risk of contamination of the sample and to protect the user (21, 22).

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments (23 to 28).

6. Diagnostic tests and investigation

6.1 Culture and microscopy

6.1.1 Specimen type

Prosthetic joint aspirate, peri-prosthetic biopsy, intra-operative specimens (debridement and retention or revision surgery), prostheses, fixation devices

Percutaneous joint aspiration

This is an important diagnostic sample for testing in both acute and chronic prosthetic joint infections. It is performed aseptically, ideally in radiology or in theatres. In acute infections, a Gram stain is useful although a negative result should not rule out the possibility of infection. In chronic infections the sensitivity of a Gram stain is less than 10% (29, 30).

A semi-quantitative white cell count on the synovial fluid is useful for differentiating inflammatory from non-inflammatory arthritides; however it is less useful at differentiating infection from inflammation (2). A total synovial cell count may be helpful in certain clinical situations (31 to 33). Cut off values for synovial fluid leucocyte count and differential cell counts for the diagnosis of prosthetic joint infection have been determined in several studies (34, 35). Leukocyte cut offs ranged from 1100 cells/µL to over 4000 cells/µL (33, 36 to 39). Leukocyte differentials ranged from 64% to greater than or equal to 80% neutrophils (33, 36 to 39). These cut off values are lower than those in cases of septic arthritis (36, 37). Specificity and sensitivity varied ranging from 82 to 98% and 84 to 97% respectively (33, 36 to 39). In patients with underlying inflammatory disease, counts may be high even in the absence of infection. When appropriate, synovial fluid should also be examined for crystals. A synovial biopsy may also be considered.

The cut offs for acute prosthetic joint infection occurring within 6 weeks of surgery, as agreed (strong consensus) at the proceedings of the international consensus meeting on periprosthetic joint infections, are as follows (35):

- synovial white blood cell count (WBC) greater than 10,000 cells/µL
- synovial neutrophil percentage (PMN%) greater than 90%

The approximate cut off for chronic prosthetic joint infection, applicable to tests taken more than 6 weeks after the most recent surgery, are as follows (35):

- synovial white blood count (WBC) greater than 3,000 cells/µL
- synovial neutrophil percentage (PMN%) greater than 80%

Broth enrichment cultures are important as the patient may have already received antibiotics and in chronic cases the number of free (planktonic) organisms may be very low. In the presence of a joint prosthesis, any organism cultured may be relevant and should be identified, have sensitivity testing performed and be reported. Many chronic infections are due to 'skin flora'. For this reason differentiating infection from contamination in a sample obtained as an aspirate is difficult. In addition the sensitivity of an aspirate in chronic infection may be poor. A peri-prosthetic tissue biopsy which can include histology could be considered.

Percutaneous biopsy

A peri-prosthetic biopsy can be obtained under ultrasound or other dynamic imaging, such as fluoroscopy. If the joint is loose, ideally this should be obtained from the bone cement interface or bone prosthesis interface. It has the advantage over needle aspiration alone, that histology, looking for neutrophils, can also be carried out if multiple biopsies can be performed.

Intra-operative biopsy

Intra-operative biopsies may be performed in the chronically infected joint either solely as a diagnostic test, as part of a debridement and retention procedure, or when a joint is being revised. Joint revision is a common procedure and usually done for aseptic loosening. However, because infection can be occult, it is advisable to take multiple samples for microbiology and histology in all cases. In some cases, where available, this can be combined with a frozen section to aid surgical decision making (40, 41).

Explanted prostheses

Explanted prostheses can be sent for microbiological investigation. They are often difficult to handle unless especially large pots are used leading to a potentially greater risk of contamination. Some laboratories sonicate the prostheses and culture the sonicate fluid. This can be done in addition to multiple samples but not to replace them.

Sonication, when used as an addition to conventional culture has been shown to improve the sensitivity of prosthetic joint infection microbiological diagnosis (42 to 44). It uses ultrasound to disrupt the bacterial biofilm on the prosthetic material. The improvement in sensitivity is most markedly seen in patients on antibiotics within 14 days prior to surgery (42, 45).

6.1.2 Pre-laboratory processes (pre analytical stage)

This section covers specific safety considerations related to this UK SMI (23, 24) (46), and should be read in conjunction with the generic safety considerations on GOV.UK.

Specimen collection, transport and storage:

For safety considerations refer to Section 2.

Collect specimens before antimicrobial therapy where possible (46).

If possible stop all antibiotics at 2 weeks prior to sampling and consider not giving routine surgical prophylaxis until after sampling (42, 47, 48).

To enable timely clinical management, samples should be processed urgently.

Swabs are to be discouraged. However if sent, swabs for bacterial and fungal culture should be placed into appropriate transport medium and transport in sealed plastic bags.

For aspirates and radiologically guided biopsies, it is usually only possible to send one sample to microbiology. In theatres, multiple (3 to 5) samples should be taken using separate instruments for microbiology. An equivalent set of samples should be taken for histology.

Specimen size should approximate 1mL.

Small volumes of synovial fluid (less than 1mL) may impede the recovery of organisms.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

Specimens should be transported and processed as soon as possible (46).

The Infectious Diseases Society of America (IDSA) guidelines recommend that specimens should be transported at room temperature, and should be processed immediately, and within a maximum of 2hr (46).

If processing is delayed, refrigeration is preferable to storage at ambient temperature (46).

Samples of fluid, pus, synovium, granulation tissue, membrane (the tissue that forms at the bone-cement or bone-prosthesis interface) and any abnormal areas should be taken, in cases where the joint is being removed. Each specimen should be taken with a separate set of instruments and should be placed into a separate specimen container. Pre-sterilised packs can be produced for this purpose. At this stage a frozen section may also be performed if available and required to decide between 1 and 2 stage exchange.

In centres where sonication is available, the prosthesis, or its components thereof, can be sent to the laboratory in a sterile watertight container.

Pre-treatment

Soft tissue samples (49, 50)

The objective should be to minimise the manipulation on the number of times any container is opened and resulting exposure of the operative sample to contamination.

It may be possible in units with high workloads of this specimen type to arrange provision and use of CE Marked leak proof container with approximately 10 glass beads and 5mL Ringer’s or normal saline to the operating theatre. It is not uncommon, however, for microbiology and histology specimen pots to be confused leading to difficulties in processing samples. Transfer of biopsies in theatres may diminish the risk of contamination during laboratory processing. In such circumstances homogenisation could be performed in the original container.

Alternatively, samples may be sent to the laboratory in CE Marked leak-proof container in a sealed plastic bag with no glass beads. Glass beads and Ringer's or saline can be added in the laboratory, maintaining asepsis diligently. Ideally processing of samples should take place in a Class II cabinet (21, 22). Homogenisation with glass beads can be performed, for example, by shaking at 250 rpm for 10 minutes in a covered rack on an orbital shaker or, alternatively, vortexing for 15 seconds (40Hz) (alternative methods of homogenisation may also be used) (41).

The diluent for the glass beads and tissue should be Ringer's or saline. Sterile molecular grade water and new universal containers should be used if direct PCR assays are planned. The volume used in the latter case should not exceed 2mL to maintain assay sensitivity.

6.1.3 Laboratory processes (analytical stage)

Specimen processing

Samples can be transferred to the laboratory using routine timescales (that is, within hours rather than minutes). There are no published comparisons or validations of various tissue processing methods in the orthopaedic setting. The method of shaking with glass beads is relatively simple and carries a low risk of contamination which has been shown experimentally to be superior to shaking in broth alone in the recovery of Bacillus spores from polymer surfaces (50). Results of a study suggest that the use of glass beads in the microbiological examination of intra-operative periprosthetic samples may indeed be a useful addition to conventional culture leading to increased microbiological diagnosis rates with relatively low contamination rates (49).

Sonication of removed components has been examined as a means of disrupting bacterial biofilm in vascular and orthopaedic prostheses(51). A considerable number of studies have now been performed comparing sonication of the prosthesis in a sterile pot to conventional cultures (18, 51 to 55). Several centres have now adopted this as routine practice (1, 56 to 58).

If available, microbiological analysis should be carried out in a Class II cabinet, using aseptic technique, to reduce the risk of contamination of the sample and to protect the user (21, 22).

Inoculate plates and broth after homogenisation of soft tissue samples and prosthesis, or directly from aspirate fluid. Inoculate each agar plate (if used) with a drop of the solution using a sterile pipette (see Q 5 – inoculation of culture media for bacteriology). Primary plates may not be required in elective revisions, in high volume units and skilled multiple site sampling.

In addition, place some of the solution into an enrichment broth. If mycobacterial cultures are required this solution can then be used to inoculate mycobacterial cultures. This is best done 24 hours after the primary plates have been examined once, to decide if decontamination of the sample is required. For information regarding incubation conditions and duration refer to B 40 – Investigation of specimens for *Mycobacterium* species.

Incubate the enrichment broth for 5 days (culture may be extended to 14 days in certain situations), examining daily for evidence of growth (35). Subculture if cloudy,

but otherwise perform a terminal subculture at 5 days. As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system for 5 days (extended to 14 days in certain situations) (21, 35, 59 to 61). Only subculture bottles which flag positive; a terminal subculture at 5 days is not required.

For the isolation of individual colonies, spread inoculum using a sterile loop.

If done, primary plates should be examined with under magnification for small-colony variants (62, 63). Care should be taken to distinguish small tissue fragments on the plate from small colonies. Small colony variants are often thymidine-dependent, if the patient has received co-trimoxazole. Such isolates may not grow well on horse blood agar due to partial lysis and release of thymidine phosphokinase from the red cells. The heating process used to produce chocolate agar destroys thymidine phosphokinase.

Optional

Specimens collected into appropriate CE marked leak proof containers should be used for microscopy and may be used for molecular techniques. Specimens for molecular testing should be processed according to manufacturer's instructions (23, 24).

6.2 Microscopy and culture

Microscopy

Gram staining in elective revision cases should not be considered for diagnosing infection as it has extremely poor sensitivity (29, 30, 35, 64, 65).

Gram staining can however be used in acute infection to distinguish between aggregates of ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria (30). These can appear as single cells or very small groups of cells. A negative Gram stain does not however rule out infection. False positive Gram stains associated with periprosthetic infections are rare, but may have severe consequences if used as the basis for treatment (29). One study investigating false positive intraoperative Gram stains in diagnosing periprosthetic infection reported a sensitivity and specificity of 9% and 99% respectively (29). Another study concluded that Gram stains have a limited application, and reported a sensitivity and specificity of 7% and 92% respectively (66).

Culture methods should include an enrichment broth. Cooked meat broth and continuous monitoring blood culture systems (CMBCS) have equivalent sensitivity, and are more sensitive than fastidious anaerobic agar plates in orthopaedic device related infection (21, 60, 67, 68).

Gram stain

Refer to TP 39 – Staining procedures

This is an insensitive procedure and not recommended for the pre or intra-operative diagnosis of chronic prosthetic joint infection.

It may however have a role in acute prosthetic joint infection especially on a purulent aspirate or surgical pus. It is important to distinguish between aggregates of

ultrasound-dislodged biofilm bacteria from other debris and contaminating bacteria. These can appear as odd single cells or very small groups of cells. A negative Gram stain does not rule out infection.

Total white cell and differential leucocyte count

Total white cell counts and differential leucocyte counts may be performed on joint aspirates.

Total white cell count

The presence of a clot will invalidate a cell count.

Perform a total cell count on the synovial fluid in a counting chamber.

Differential leucocyte count – Toluidine blue/Methylene blue stain (Wright stain)

(refer to TP 39 – Staining procedures)

Note: When a particle counter has been validated for use with cells in this context then it may be used (69).

Differentiating between polymorphonuclear leucocytes and mononuclear leucocytes may be performed in 2 ways:

- **Counting chamber method: recommended for lower WBC counts.**

  a) Non- or lightly-blood stained specimens

  - stain the fluid with 0.1% stain solution such as toluidine, methylene or nile blue. This stains the leucocyte nuclei thus aiding differentiation of the cells
  - the dilution factor must be considered when calculating the final cell count
  - count and record the numbers of each leucocyte type
  - express the leucocyte count as number of cells per litre

  b) Heavily bloodstained specimens

  - dilute specimen with WBC diluting fluid and leave for 5 minutes before loading the counting chamber. This will lyse the red blood cells and stain the leucocyte nuclei for differentiation
  - count and record the number of each leucocyte type: the dilution factor must be considered when calculating the final cell count
  - express the leucocyte count as number of cells per litre

- **Stained method**

  Recommended for very high WBC counts where differentiation in the counting chamber is difficult

  - prepare a slide from the centrifuged deposit or cytospin preparations as for the Gram stain but allow to air dry

- fix in alcohol and stain with a stain suitable for WBC morphology

  Note: Heat fixation distorts cellular morphology

- count and record the number of each leucocyte type as a percentage of the total

6.2.1 Laboratory processes (analytical stage)

Culture

Sample preparation

For safety considerations refer to Section 2.

Inoculate each agar plate with sample using a sterile pipette Q 5 – Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

Duration of culture

Traditionally orthopaedic samples have been cultured for up to 5 days. More recently evidence suggested that incubation of up to 14 days may be necessary to isolate less virulent organisms such as propionibacteria and diphtheroids (35, 70 to 74). The methods described omit any early subculture unless broths are cloudy. Visual inspection of broth media is not very accurate; many earlier positives may have been missed and time to detection may depend on how frequently cultures are examined (75). Some broth enrichment methods require incubation for 14 days, however in a protocol based on vortexing with sterile beads and enrichment in cooked meat broth with terminal subculture after 5 days, the sensitivity of the broth enrichment after 5 days was almost equivalent to the sensitivity obtained with 2 blood cultures (aerobic and anaerobic) despite the lower inoculum used for the broth. If a set of aerobic and anaerobic bottles are used for the enrichment, an analysis of receiver-operator characteristics (ROC) has demonstrated that there is no need for incubation times exceeding 5 days (21, 60). Full automation using CMBCS bottles suggests that greater than 98% of significant results have flagged within 3 days (60).

This UK SMI therefore recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system methods (51, 60, 61). However, in cases of suspected prosthetic joint infection, with low virulence organisms, or where preoperative cultures have failed to show growth and the clinical picture is consistent with prosthetic joint infection, culture may be extended to 14 days (35, 59, 76).

Specimen processing

Table 1: Culture media, conditions and organisms

Clinical details and/or conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
All clinical conditions (Primary plates may not be needed in elective revisions, in high volume units and skilled multiple site sampling)	All specimens	Blood agar and Chocolate agar	35 to 37	5 to 10% CO_2	40 to 48hr	Daily	Staphylococci Streptococci Enterococci Enterobacteriaceae Fastidious Gram negatives Pseudomonads Yeast Mould
		FAA	35 to 37	Anaerobic	5d	3d and 5d	Anaerobes

Clinical details and/or conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
		Fastidious anaerobic, cooked meat broth or equivalent. *Subculture if evidence of growth, or at day 5 on plates as below: or **blood culture for CMBCS. (aerobic and anaerobic bottles(68)) Subculture when flags positive on plates as below:	35 to 37	Air	5d *** or up to 5d ***	Daily	Any
		Blood agar	35 to 37	Anaerobic	40 to 48hr	Daily	Any anaerobes
		Chocolate agar	35 to 37	5 to 10% CO_2	40 to 48hr	Daily	Any
Fungal infection suspected(77)	All specimens	Sabouraud agar (slopes)	28 to 30	Air	14d	Daily	Yeast and mould

Clinical details and/or conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Optional molecular techniques							

Clinical details and/or conditions	Specimen	Molecular technique	Instructions	Target organism(s)
All clinical conditions	Bone marrow	NAAT	Follow manufacturer's instructions	Any organism

Always consider other organisms such as *Mycobacterium* species (B 40 – Investigation of specimens for *Mycobacterium* species), fungi and actinomycetes.

* Broths should be examined periodically (ideally daily) and subcultured if there is evidence suggestive of growth. Terminal subcultures should be performed at 5 days.

** Blood culture subcultures should be performed when the bottle flags positive. A terminal subculture at 5 days is not required (51, 60).

*** Incubation may be extended if clinically indicated. For example in cases of suspected prosthetic joint infection with low virulence organisms, or where preoperative cultures have failed to show growth, and the clinical picture is consistent with prosthetic joint infection, culture may be extended to up to 14 days.

Identification

Refer to individual UK SMIs for organism identification.

Minimum level of identification in the laboratory

Actinomycetes	genus level ID 15 – Identification of anaerobic *Actinomyces* species
Anaerobes	genus level ID 14 – Identification of anaerobic cocci ID 8 – Identification of *Clostridium* species ID 25 – Identification of anaerobic gram negative rods
β-haemolytic streptococci	Lancefield group level or species level
Other streptococci	species level
Enterococci	species level
Enterobacteriaceae	species level
Yeast and mould	species level
Haemophilus species	species level
Pseudomonads	species level
S. aureus	species level
Staphylococci (not aureus)	to coagulase negative Staphylococci or species level (if multiple samples)
Mycobacterium species	B 40 – Investigation of specimens for *Mycobacterium* species

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: No organism should be considered to be a contaminant until cultures on all samples are concluded. Identification to species level and/or an extended antibiogram is normally necessary to detect whether isolates from multiple samples are indistinguishable.

Note: Laboratories should save all samples and isolates for at least 2 weeks in case further work (unusual organisms, molecular studies or further sensitivities) is required.

Automation(60)

Some laboratories with a significant number of orthopaedic device related samples have opted to use automation using CMBCS to reduce labour and early subculture of culture broths (21, 67).

6.3 Rapid techniques

6.3.1 Serology

Serological techniques used for diagnosis of prosthetic joint infection have been studied in the research setting but have not yet been found to be of practical clinical benefit. The problem tends to be with specificity (78).

6.3.2 Molecular methods

Nucleic acid amplification techniques (NAAT) (36, 79 to 86)

NAAT – Nucleic acid amplification techniques (for example, PCR) for the identification of bacteria, fungi, parasites and viruses from clinical samples have been shown to be highly specific and sensitive. PCR targets conserved genes of the genome, and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short timeframe particularly if multiplex real-time PCR is used.

NAAT including PCR, 16s rRNA gene PCR and PCR-electrospray ionization (ESI)/MS have been developed as a means of rapid, sensitive identification of organisms associated with prosthetic joint infection (79 to 82). PCR has been shown to be more sensitive than convention culture for the isolation of some fastidious organisms for example *Kingella kingae*, and PCR–hybridization after sonication has been shown to improve diagnosis of implant related infections (56, 87).There are however some issues with NAAT analysis. A lowered sensitivity may be observed due to the small volume of samples processed, in some cases there may be interference with human DNA originating from the tissue samples, and antibiotic susceptibility information is not available (17, 88).

Future developments may include the coupling of broad range PCR with next generation sequencing allowing analysis of the total microbiome associated with orthopaedic implant associated infections (83).

6.3.3 MALDI-TOF mass spectrometry

Recent developments in identification of bacteria, and fungi include the use of protein profiles obtained by Matrix assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectrometry (89). Mass peaks achieved by the test strains are compared to those of known reference strains. It is possible for an organism to be identified from an isolate within a short time frame and it is increasingly being used in laboratories to provide a robust identification system (79).

Limited data is available on the use of MALDI-TOF MS in orthopaedic implant associated infection diagnosis; however, one study has shown that MALDI-TOF MS may be useful diagnostic tool for identifying isolates from patients with orthopaedic implant associated infections, providing reliable species level identification which may be used to differentiate between contaminates and pathogens (89, 90),(91).

6.4 Post-laboratory processes (post analytical stage)

6.4.1 Culture

Interpreting and reporting results

Following results should be reported:

- all organisms
- absence of growth

Intra-operative samples interpretation: 2 or more samples with an indistinguishable organism is indicative of a prosthetic joint infection.

Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Note: Due to extended incubation in certain situations, some final reports may not be available until greater than 14 days.

Also, report results of supplementary investigations.

Supplementary investigations: B 40 – Investigation of specimens for *Mycobacterium* species

6.4.2 Microscopy

Interpreting and reporting results

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Gram stain

Report on organisms detected.

Note: Not to be used for diagnosis of chronic prosthetic joint infection.

White cell count

Report numbers of WBCs x 10^6 per litre.

Differential count

Report mononuclear leucocytes as percentage of the total WBCs.

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Thresholds for white blood cell count and neutrophil percentage(35)

	Approximate cut off for acute PJI less than or equal to 6 week after most recent surgery	Approximate cut off for acute PJI greater than 6 week after most recent surgery
Synovial white blood cell count (WBC)	greater than 10,000 cells/μL	greater than 3,000 cells/μL
Synovial neutrophil percentage (PMN%)	greater than 90%	greater than 80%

6.4.3 Molecular

Report results as per manufacturer's instructions:

- detected
- not detected

6.5 Technical information and limitations

6.5.1 Sonication

Gram positive bacteria have been found to be resistant to the effect of ultrasound; Gram negative organisms may be more susceptible (58). The effect of sonication on fungi and *Mycobacterium* species is unknown.

There may be the potential for contamination of sonication fluid during collection or specimen processing. Particular care needs to be taken during opening and closing the container lid, to ensure that no contact is made with inner surface of the lid. Contamination is usually indicated by low counts of environmental bacteria (9, 36, 92).

6.5.2 Small colony variants

Where primary plates are used they should be examined under magnification as small colony variants of staphylococci may be isolated from deep samples (63). Such small colonies may only become evident on prolonged culture (62). Thymidine dependent auxotrophs usually do not grow on blood agar and have atypical colonial appearance resembling haemophili or streptococci on chocolate agar (93). The true prevalence and clinical relevance of small colony forms in prosthetic joint infection is unclear (63, 94).

6.5.3 Contamination

Repetitive subculture from the enrichment broth during incubation may lead to contamination; the use of continuous monitoring blood culture bottles reduces this risk (21).

6.5.4 Effect of antibiotic use

In cases where a prosthetic joint is chronically painful, functioning poorly and/or loose, an elective revision will be performed. Patients should be off antibiotics for at least 2 weeks (42, 47, 48). One study comparing the culture of samples obtained by sonication of the prostheses and conventional periprosthetic-tissue culture has shown

that sensitivity of both culture methods is reduced in patients receiving antimicrobial therapy within 14 days before surgery (42). The effect of a single dose of antibiotic on the sensitivity of microbiological culture is unknown and, where the suspicion of infection is low, timely administration of prophylactic antibiotics is paramount (for example in the 30 to 60 minutes prior to skin incision) (94). The timing of prophylactic antibiotics is a risk-benefit decision.

Revision arthroplasty involves the removal of a prosthetic joint and debridement followed by re-implantation. Re-implantation may or may not occur during the same operation. In patients with a known chronically infected joint or one where evidence of infection (purulence) is found intra-operatively, the preferred option in many centres is to remove the joint and do a thorough debridement without immediate re-implantation. This is termed the ‘first stage’ of a 2 stage revision. In some centres in selected cases however, one-stage revision is performed even in the presence of infection (95, 96).

7. Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

Extensive antibiograms (including rifampicin) on multiple isolates are required (32, 51, 60). It is important to include a wide range of antibiotics particularly for those patients who may require prolonged oral treatment with biofilm active drugs. These antibiotics are not usually included in the common first line antimicrobials tested in most laboratories. For Gram positive organisms these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracycline, quinolone, co-trimoxazole, fusidic acid and linezolid.

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

8. Notification to PHE or equivalent in the devolved administrations

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within 7 days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within 7 days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily

report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue(97, 98),(99 to 102).

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) and Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland (99, 100)

9. Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

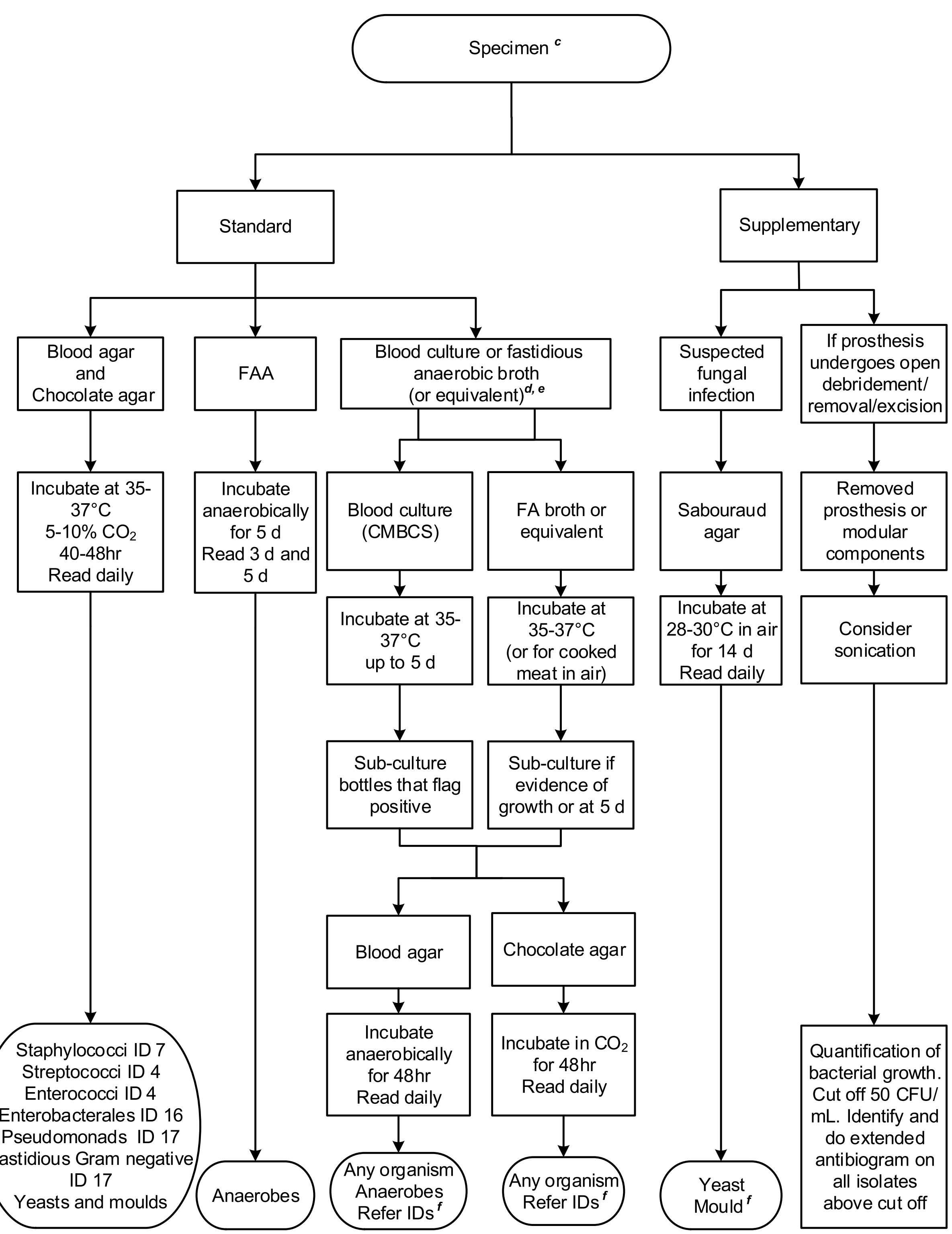
Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

- England and Wales
- Scotland
- Northern Ireland

Notes: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix: Investigation of orthopaedic implant associated infections by culture[a, b]

A text description of this algorithm is provided with this document on the UK SMI B 44: investigation of orthopaedic implant associated infections webpage.



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Footnotes

a) Laboratories should save all samples and isolates for at least 2 weeks in case further work (unusual organisms, molecular studies or further susceptibilities) is required.

b) Microbiological tests may not be required for synovial biopsy specimens if proceeding straight to revision or removal.

c) Mycobacterial cultures if any clinical suspicion such as ethnic origin, plus previous unexplained culture negative samples, granulomas on histology, chest-X-ray findings, and previous history of TB (see B 40 – Investigation of specimens for *Mycobacterium* species).

d) As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system (CMBCS) for up to 5 days. Terminal subculture is not required.

e) In cases of suspected prosthetic joint infection, with low virulence organisms, or where preoperative cultures have failed to show growth and the clinical picture is consistent with prosthetic joint infection, culture may be extended to 14 days (35).

f) Interpretation of intra-operative samples: 2 or more samples with an indistinguishable organism are a positive microbiology result.

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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 44 Investigation of orthopaedic implant associated infections

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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A50711295

1st Consultation 09/08/2013 – 01/11/2013

Version of document consulted on – B 44dk+

Proposal for changes

Comment number	1		
Date received	28/10/2013	**Lab name**	Oxford
Section	Several		
Comment			
a. Please delete Dr Ivor Byren and Dr Tony Berendt from acknowledgements section (unless they have contributed separately). b. i. Risk factors for Infection: suggest patient factors not patient co-morbidities. ii. Diagnosis: put Plain X-rays are performed to look for early loosening, fracture or other pathology. iii. Diagnosis: Put a joint aspirate for cell count, culture and histology (using ultrasound....) c. Percutaneously biopsy (heading) should be percutaneous biopsy. d. Intra-op biopsies - put to aid surgical decision making. e. Automation: can quote Minassian abstract below. f. Duration of culture: although we discussed and thought 5 days culture would be OK, we have locally decided on 10 days for now subject to evaluation again in the future. The Minassian data is fairly convincing for shortening but colleagues want to be cautious as clinical stakes are high. This may be sensible to recommend in this guidelines too? Say 7-10 days or up to 10 days. Reference for Minassian is below. This duration would have to also be inserted in the soft tissue homogenate section and table 2.5.3 if this is what you decide. g. Footnotes: d) I would say use separate sterile instruments for all samples as it applies across the board for PJI. The SMI looks very good.			
Evidence			
BACTEC for diagnosis of prosthetic joint infections: easy, cheap and fast Angela Minassian, Robert Newnham, Elizabeth Kalimeris, Philip Bejon, Bridget Atkins, Ian CJW Bowler. Abstract, IDSA ID Week, San Francisco 2013. http://www.icaaconline.com/php/icaac2013abstracts/data/papers/2013/H/2013_H-1000.htm			
Financial barriers			
No.			
Health benefits			

No.	
Recommended action	a. **ACCEPT** Text updated. b. i. **ACCEPT** Text updated. ii. **ACCEPT** Text updated. iii. **ACCEPT** Text updated. c. **ACCEPT** Text updated. d. **ACCEPT** Text updated. e. **ACCEPT** Abstract will be included in the references. Final paper has been published. Recommendation up to 5 days. Minassian AM, Newnham R, Kalimeris E, Bejon P, Atkins BL, Bowler IC. Use of an automated blood culture system (BD BACTEC) for diagnosis of prosthetic joint infections: easy and fast. BMC Infect Dis 2014;14:233. f. **PARTIAL ACCEPT** Following discussion at the working group, 5 day incubation was agreed. g. **NONE** Flowchart updated post consultation footnote no longer included.

2nd Consultation 15/09/2014 – 13/10/2014

Version of document consulted on – B 44dv+

Proposal for changes

Comment number	1		
Date received	22/09/2014	**Lab name**	Bone Infection Unit, Oxford
Section	Explanted prosthesis		

Comment	
Would delete. It may reduce the number of tissue samples required to 3-4 as there is no evidence for this and in fact we have some preliminary data, presented at the European Bone and Joint Infection Society in Sept 14, to show that diagnostic sensitivity would be reduced if you reduce the number of specimens, even if done in combination with sonication.	
Evidence	
Oral presentation at EBJIS 2014 F031 Sonication for diagnosis of non-prosthetic-joint orthopaedic infections Brent AJ, Dudareva M, Colledge R, Figtree M, Newnham R, Bejon P, Woodhouse A, Taylor A, McNally MA, Atkins BL.	
Do you receive and process swabs for the investigation of orthopaedic implant associated infections? Please comment.	
Swabs not indicated.	
Which method of homogenisation do you use (eg glass beads, vortex etc)? Please record details below.	
Glass (Ballotini) bead.	
Do you, or would you, prefer the use of continuous monitoring blood culture bottles, or cooked meat broth for enrichment? Please comment.	
Continuous monitoring.	
Recommended action	**ACCEPT** Sentence removed.

Comment number	2		
Date received	10/10/2014	**Lab name**	Royal Liverpool University Hospital
Section	4.5: Culture and Investigation		
Comment			
a. We were particularly interested in the recommendation to use either standard enrichment broth or blood culture system as enrichment. Our current practice is to use Robinsons Broth however we feel this is time consuming and labour intensive compared to using a blood culture system. However we were concerned that our current blood culture bottles would not be compatible with inoculation of homogenised tissue. b. We appreciate the recommendation to use a hand lens to assay small-colony variants but feel this is challenging given the move to automation (we are using a Kiestra system).			
Do you perform cell counts on synovial fluid? If so, do you think a dilution step should be added to the cell count method in this SMI? Please comment.			

Yes if required but not routinely. We do not feel a routine dilution step would be beneficial.	
Do you receive and process swabs for the investigation of orthopaedic implant associated infections? Please comment.	
Yes, but in conjunction with other tissue samples (ie the swab is not the sole sample received for diagnosis of PJI).	
Which method of homogenisation do you use (eg glass beads, vortex etc)? Please record details below.	
We use Ballotini glass beads for hard tissue and disposable pestle and mortar for soft tissue infection.	
Do you, or would you, prefer the use of continuous monitoring blood culture bottles, or cooked meat broth for enrichment? Please comment.	
We currently use Robinsons cooked meat broth but are interested and keen to explore the potential to move to a continuous monitoring system - see notes above.	
Recommended action	a. **PARTIAL ACCEPT** The following text has been added: *'Sterile, needleless syringes and blood transfer devices are commercially available which may be used for the aseptic transfer of sample homogenate into blood culture bottles.'* b. **ACCEPT** Text changed to 'under magnification'.

Comment number	3		
Date received	15/10/2014	**Lab name**	PHE HCAI & AMR Dept, CIDSC
Section	Risk Factors for Infection, Pathogenesis and microbiology, Diagnosis, Sample types		
Comment			

a. Risk factors
   i. The benefit of laminar air flow systems in theatre has become somewhat controversial given results of recent studies. As such, we would recommend removing or replacing this with a more general statement on the importance of controlling the theatre environment.
   ii. English national surveillance could helpfully be cited here alongside the SSI rates (or ECDC data which includes UK data):
   Public Health England. Surveillance of surgical site infection in NHS hospitals in England 2012/13. Public Health England **2013**. Available at: https://www.gov.uk/government/publications/surgical-site-infections-ssi-

	surveillance-nhs-hospitals-2012-to-2013 iii. The excess risk according to NNISS risk score (now NHSN) could be described according to the components of the score for ease of reading by individuals unfamiliar with it (ie duration of surgery, degree of wound contamination and patient's preoperative health). The following more recent UK paper could also be cited to illustrate the impact of these factors on SSI risk: Lamagni T. Epidemiology and burden of prosthetic joint infections. *J Antimicrob Chemother* 2014 69 (9): i5-i10 doi: 10.1093/jac/dku247. iv. Please note the NHSN risk score encompasses patient and surgical factors (not just patient as described in the SMI). v. The importance of surveillance as a means to prevention could be added to this section or elsewhere, along with the NICE guideline and accompanying Quality Standards for prevention of SSI. National Institute for Health and Clinical Excellence (2008) Surgical Site Infection: Prevention and Treatment of Surgical Site Infection. London: NICE. https://www.nice.org.uk/guidance/cg74 National Institute for Health and Clinical Excellence (2013) Quality Standards for Surgical Site Infection QS49 http://publications.nice.org.uk/surgical-site-infection-qs49/list-of-quality-statements b. Pathogenesis and microbiology PJI can arise from post-op contamination of the wound also. c. Diagnosis Whilst perhaps self-evident, you might wish to consider making specific recommendation that clinical samples are ideally taken prior to antibiotic treatment. d. Sample types *Duration of culture* There may be a typo here "cultures" vs "cultured".
Recommended action	a. i. **PARTIAL ACCEPT** Text updated, reference to laminar flow removed. ii. **ACCEPT** Reference to PHE Surveillance of surgical site infections in NHS hospitals in England 2013/2014 included. iii. **ACCEPT** Text updated. iv. **PARTIAL ACCEPT** Lamagni 2014 reference added to text.

	v. **ACCEPT** Text updated and references added. vi. **ACCEPT** Text updated and references added. b. **ACCEPT** Text updated. c. **ACCEPT** Text updated in section 2.2. d. **ACCEPT** Text updated.

Comment number	4		
Date received	23/10/2014	**Lab name**	The Royal London Hospital
Section	See below		
Comment			

a. Percutaneous joint aspiration (P9)

   Change to: Acute prosthetic joint infection occurring within six weeks of surgery, as agreed (strong consensus) at the proceedings of the international consensus meeting on periprosthetic joint infections are as follows:

b. Percutaneous biopsy (P9)

   Change to: multiple biopsies can be performed.

c. Sampling (P10)

   Change to: In centres where sonication is available, the prosthesis, or its components thereof, can be sent to the laboratory in a sterile watertight container.

d. Sample processing (P10)

   It is surprising that the evidence is limited regarding comparisons and validation of tissue processing. Have all the studies been examined. Reference 27 is rather narrow. Are there any other studies other than ref 28 regarding glass beads in a broth to determine contamination?

e. Microscopy and culture (P10)

   Details on performance characteristics would be useful for quantification.

f. Duration of culture (P11)

   On balance this seems A reasonable approach as the lab may otherwise end up with prolonged incubation for non-infected prosthetic samples.

<table>
<tr><td colspan="2">

i. However where is the evidence for direct culture for these tissues? Many labs and indeed some literature suggest keeping plates for 5 days.

Pragmatically a combination of plate culture and enrichment broths can increase yield and also shed light for determining clinical significance eg for propionibacteria.

ii. Is there other evidence for 5 days other than ref 36 and 38?

g. Nucleic acid amplification techniques (NAATs) (P12)

Is there a better example of a fastidious organism? It is not a common cause of prosthetic joint infections but causes septic arthritis endocarditis in children.

h. Contamination (P13)

Change to: Repetitive subculture from the enrichments broth during incubation may lead to contamination; the use of continuous monitoring blood culture bottles reduces the risk of contamination.

i. Effect of antibiotic use (P13)

‘Patients should be off antibiotics for at least two weeks.’ What is the evidence for this?

j. Specimen processing/procedure (P16)

In this section, we couldn’t find a method for sonication. Should sonication occur in theatre or in the lab?

k. 4.3.1 Pre-treatment (P17)

‘As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system for up to 5 days.’

Maybe 14 days in some situations as discussed on page 11.

l. 4.3.2 Specimen processing (P17)

i. ‘This is best done 24hr after the primary plates have been examined once, to decide if decontamination of the sample is required.’

Length of incubation not mentioned eg 5 days in a moist environment.

ii. ‘As an alternative to enrichment broth, samples may be cultured in an automatic continuous monitoring blood culture system for 5 days.’

Need to mention extending 14 days in some situations.

</td></tr>
<tr><td>**Recommended action**</td><td>

a. **ACCEPT**

Text updated.

b. **ACCEPT**

Text updated.

c. **ACCEPT**

Text updated.

d. **ACCEPT**

A literature search was undertaken. No additional

</td></tr>
</table>

	references were identified. e. **ACCEPT** Text regarding sensitivities and specificity included. References including information on sensitivity, specificity and positive and negative predicative values added. f. i. **PARTIAL ACCEPT** Direct plates were included following consensus decision by the Bacteriology Working Group. It was acknowledge that primary plates may not always be required. The following text has been added to section 4.3.2 in line with section 4.5.3: *'Primary plates may not be required in elective revisions, in high volume units and skilled multiple site sampling'.* ii. **ACCEPT** Additional references regarding 5 day incubation have been included. g. **NONE** The group felt that *Kingella kingae* was an appropriate example of a fastidious organism. h. **ACCEPT** Text updated. i. **PARTIAL ACCEPT** Cessation of antimicrobial therapy 14 days prior to surgery is discussed in various papers as best practice. Additional references have been added to the text and the text has updated to include a study by Trampuz et al 2013. *'One study comparing the culture of samples obtained by sonication of the prostheses and conventional periprosthetic-tissue culture has shown that sensitivity of both culture methods is reduced in patients receiving antimicrobial therapy within 14 days before surgery'* Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007;357:654-63 j. **PARTIAL ACCEPT** Sonication is an optional method for processing of the prosthesis. Information is included in the introduction (sample processing section) which states: *'In centres where sonication is available, the prosthesis,*

	or its components thereof, can be sent to the laboratory in a sterile watertight container' k. **ACCEPT** Text updated. l. i. **PARTIAL ACCEPT** Link to B 40 – Investigation of *Mycobacterium* species added. ii. **ACCEPT** Text updated.

Comments received outside of consultations

Comment number	1		
Date received	04/03/2013	**Lab name**	Salford Royal Foundation Trust
Section			
Comment			
a. Fastidious anaerobic broth: It is stated that when broth becomes cloudy it should sub-cultured. Once they have been sub-cultured are they re-incubated for the full 5 days? b. Sub-culture plates from cloudy broths: if bacterial growth is indicated at 24hr/48hr is the incubation of broth discontinued. If no bacterial growth I would assume the broth continues its incubation period. c. Primary culture: If the primary culture plates indicate bacterial growth at 24/48hr would you subculture the broth and then discontinue its incubation period of 5 days or would it be re-incubated.			
Recommended action	a. **ACCEPT** Text updated for clarity: '*Broths should be examined periodically (ideally daily) and subcultured if there is evidence suggestive of growth. Terminal subcultures should be performed at 5 days'.* b. **NONE** Each line of investigation should continue until it is complete. c. **NONE** Each line of investigation should continue until it is complete.		

Comment number	2		
Date received	06/03/2013	Lab name	Belfast Trust
Section			
Comment			
What was the rationale behind suggestion to subculture a Sabouraud agar plate for 14 days? Unable to find a reference or a paper to support this.			
Recommended action	PARTIAL ACCEPT 14 day incubation of SAB agar/slopes is consistent with other recently issued UK SMIs and is based on recommendations of the UK Clinical Mycology Network. An additional reference has been included in the media table which concludes that an incubation of 2 weeks a sufficient incubation for the isolation of most yeasts and moulds from clinical samples. Bosshard PP. Incubation of fungal cultures: how long is long enough? Mycoses 2011;54:e539-e545.		

Comment number	3		
Date received	01/07/2013	Lab name	MSTAG
Section	a. General comments b. 2.4.1 c. 2.5.3 cooked meat broth or equivalent d. 2.5.3 subculture when cloudy or at day on plates as below e. 2.5.3 blood culture for CMBCS f. 2.5.3 g. 2.5.3 blood agar for anaerobes h. 2.5.3 i. 3.2.1 j. Appendix 1 k. Appendix 1 l. Appendix 2 m. Footnotes a n. Footnotes d		
Comment			
a. It was felt that there were more changes than detailed in the amendment section from the one issued last year.			

<table>
<tr><td colspan="2">
b. The laboratory would not always know if the sample was pre-or intra-operative.
c. Inconsistent with B 42.
d. It was felt that relying on “cloudiness” as a marker for sub-culturing was un-scientific.
e. Pair of bottles or just one-paediatric for example (1 suggestion of anaerobic bottle only). How do you get tissue in bottles as most have sealed caps.
f. If subculture @14 days and then onto Sab for further 14 days-this could lead to a 28 day TAT.
g. No FAA on primary or subculture, inconsistent with other SMIs, it was felt that FAA be added as a minimum standard.
h. Fungal culture section unclear.
i. Culture reporting time, 16h-14d-not possible if Sab is on subculture and takes further 14 d.
j. Gram-record presence of crystals-not thought to be a valid technique as this is carried out by polarising microscopy.
k. Sab subculture has comment incubate in NO_2.
l. A lot of the clinical details would not be known in the lab.
m. “sensitivities”? should be susceptibilities.
n. Not relevant to a laboratory SMI.
</td></tr>
<tr><td>Recommended action</td><td>
a. ACCEPT
The amendment table will be updated fully before issue.
b. NONE
This information should be requested via the user manual.
c. ACCEPT
Table updated to 5 day incubation.
d. ACCEPT
Text updated to
‘Subcultures should be examined periodically (ideally daily) and subcultured if there is evidence suggestive of growth. Terminal subcultures should be performed at 5 days’
e. ACCEPT
Section 4.5.3 updated to reflect that both aerobic and anaerobic bottles are required.
f. NONE
This is correct. No action required.
g. ACCEPT
FAA added to standard media in section 4.5.3 and
</td></tr>
</table>

	Appendix 1. h. **ACCEPT** Table updated. i. **ACCEPT** The following text has been added to section 5.2.1: ***'Note:** Due to extended incubation in certain situations, some final reports may not be available until >14 days'* j. **ACCEPT** Text removed. k. **ACCEPT** Table update to '*anaerobically*'. l. **NONE** This information should be requested via the user manual. m. **ACCEPT** Text updated. n. **NONE** Flowchart updated following consultation. Footnote no longer included.

Comment number	4		
Date received	02/08/2013	**Lab name**	BIA
Section	a. Introduction, management, paragraph 3. b. Section 2.7 c. Section 3.4		
Comment			
a. Care with terminology. As you describe, there is increasing evidence that in certain situations, prosthesis can be removed, debrided and a second prosthesis implanted. This can be referred to as a 2-stage procedure undertaken at the same operation. b. I think it is important to be explicit about critical need to perform rifampicin sensitivity (and to a lesser extent, a wider range of agents like linezolid) more or less routinely, particularly if 2-stage procedure at one operation is being considered. c. Consider reference to antimicrobial stewardship as well as clinical indications.			
Evidence			
a. I have heard the above terminology at conferences (eg Tony Berendt)			

b. CID 2013:56 1st Jan. Osmon DR et al. Diagnosis and management of prosthetic joint infection: Clin pract guidelines by IDSA.	
Recommended action	a. **ACCEPT** Text updated to include the possibility of a two stage procedure being completed in one operation. *'Revision arthroplasty involves the removal of a prosthetic joint and debridement followed by re-implantation. Re-implantation may or may not occur during the same operation.'* b. **ACCEPT** Text updated to 'Extensive antibiograms (including rifampicin) are required.' and reference assessed and added to section 4.7. In addition the following text from B42 – Investigation of bone and associated tissue has been inserted: *'It is important to include a wide range of antibiotics particularly for those patients who may require prolonged oral treatment with biofilm active drugs. These antibiotics are not usually included in the common first line antimicrobials tested in most laboratories. For Gram positive organisms these may include a teicoplanin MIC plus antibiotics such as rifampicin, tetracyclines, quinolones, co-trimoxazole, fusidic acid, linezolid, quinupristin/dalfopristin and others.'* c. **ACCEPT** Text updated to: *'Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.'*

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	29/09/2014	**Lab name**	PHW

UK Standards for Microbiology Investigations

Screening for *Neisseria meningitidis*

NICE accredited
www.nice.org.uk/accreditation

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 51 | Issue no: 2 | Issue date: 12.03.14 | Page: 1 of 18

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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 6

SCOPE OF DOCUMENT 8

INTRODUCTION 8

TECHNICAL INFORMATION/LIMITATIONS 10

1 SAFETY CONSIDERATIONS 11

2 SPECIMEN COLLECTION 11

3 SPECIMEN TRANSPORT AND STORAGE 11

4 SPECIMEN PROCESSING/PROCEDURE 12

5 REPORTING PROCEDURE 13

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 14

APPENDIX: SCREENING FOR *NEISSERIA MENINGITIDIS* 15

REFERENCES 16

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	3/12.03.14
Issue no. discarded.	1.2
Insert Issue no.	2
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Name changed to 'Screening for *Neisseria meningitidis'* from 'Screening for meningococci'. Minor textual and formatting changes.
Scope.	Type of specimen - Naso and Pernasal swabs removed, Nasopharyngeal swabs added. Scope - expanded to include when to screen for *Neisseria meningitidis*. Hyperlinks to other relevant SMI added.
Introduction.	Updated to include routes of transmission and risk factors, PHE and NICE guidelines, and information regarding serogroups. Updated carriage, spectrum of disease and epidemiology sections.
Safety considerations.	Restructured and reworded in line with new template. Safety consideration statements regarding *N. meningitidis* updated; processing of samples can be carried out at containment level 2. Standard safety references have been reviewed

	and updated.
Referral to Reference Laboratories.	Text updated, links for Scotland and Ireland added.
Notification to PHE or equivalent in the devolved administrations.	Reference updated, reference to Northern Ireland added.
Appendix 1.	Addition of Appendix 1 – Flowchart.
References.	References reviewed and updated.

Amendment No/Date.	2/01.08.12
Issue no. discarded.	1.1
Insert Issue no.	1.2
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. The term “CE marked leak proof container” is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to the Directive itself EC[1,2]. Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives
http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2014). Screening for *Neisseria meningitidis*. UK Standards for Microbiology Investigations. B 51 Issue 2. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

Type of Specimen

Oropharyngeal swabs, nasopharyngeal swabs

Scope

This SMI describes the investigation of swabs for the presence of *Neisseria meningitidis.*

Screening for *Neisseria meningitidis* (the meningococcus) should be performed when investigating a suspected case of meningococcal disease, for screening contacts of a case, and in outbreak situations to determine the extent of carriage and/or the need for prophylaxis.

B 5 - Investigation of Nose Swabs, B 9 - Investigation of Throat Swabs and ID 6 - Identification of *Neisseria* species are recommended for additional background information.

This SMI should be used in conjunction with other SMIs.

Introduction

Neisseria meningitidis forms part of the normal nasopharyngeal flora. Person to person transmission is the only known route of acquisition and usually occurs via aerosol droplets or secretions from the upper respiratory tract of an asymptomatic carrier or a close contact with invasive meningococcal disease[3]. The majority of cases (97%) of meningococcal disease which occur in the UK are sporadic, close contacts of a case are however recognised to be at an increased risk of infection[4]. To prevent onward transmission of virulent meningococci, prophylaxis (antibiotic chemoprophylaxis and vaccination if appropriate) is recommended for such contacts. The aim is to eliminate carriage of the virulent organism from the case's immediate social network. PHE recommends that nasopharyngeal swabs should be collected from all suspected cases, and the request form should specify that *N. meningitidis* is being sought[4-7]. NICE guidelines for the management of bacterial meningitis do not however recommend the use of throat swabs for the investigation of meningococcal disease in children under 16[8]. Management of outbreaks of meningococcal disease and prophylaxis is usually led by the consultant in communicable disease control (CCDC) or consultant in public health medicine (CPHM)[4].

Characterisation of the causative organism is an important consideration in outbreak management, as it determines whether cases may be related and whether vaccination of contacts may be necessary. The use of intravenous antibiotics in the community prior to hospital admission may decrease the yield of *N. meningitidis* from blood and CSF samples, nasopharyngeal swabs are less affected by prior antibiotic therapy and have been shown to yield *N. meningitidis* in 40-50% of clinical cases[4]. Confirmation of cases by non-culture (molecular) methods does not provide isolates for typing and determination of antimicrobial susceptibilities. Isolation of the organism from diagnostic or screening swabs from cases and close contacts may therefore be necessary for strain identification. Typing is important for outbreak investigations and surveillance, for the national serogroup C meningococcal vaccination programme and for detection of vaccine failures[9].

Carriage

N. meningitidis is carried on the posterior pharyngeal wall and can be detected from oropharyngeal or nasopharyngeal swabs[10]. Specimens for meningococcal screening are from two types of individuals: those infected and who may have been treated with antibiotics; and untreated asymptomatic contacts of the index case. Oropharyngeal swabs (sampling the posterior pharyngeal wall through the mouth) are ideal, but nasopharyngeal swabs (although they may be difficult to obtain) are also acceptable.

The carriage rate in the general population has been estimated to be around 10%[11]. This may be substantially higher in teenagers (25%) and young adults (32%), probably as a result of increased social activities leading to inhalation of infected respiratory secretions and by direct contact (kissing)[3,11]. Carriage rates may also be higher in close contacts of a case, in closed or semi-closed communities such as military establishments and university students, during mass public gatherings (eg the Hajj pilgrimage) and particularly during outbreaks[12]. The risk of carriage also increases with damage to the nasopharyngeal mucosa from smoking (and passive smoking) and from co-infection with influenza and *Mycoplasma* species[11]. Chemoprophylaxis should be offered to close contacts of a case who have had prolonged close contact (eg those in a household setting), and to those who have had transient close contact but who have been exposed to large droplets or secretions from the respiratory tract at the time of case admission to hospital[4]. Where there is more than one case the decision on when to extend prophylaxis will be taken by the CCDC.

Spectrum of Disease

Infection with *N. meningitidis* produces a wide spectrum of disease manifestations ranging from a mild illness with transient fever and bacteraemia to fulminant meningococcal sepsis characterised by a rapidly progressive, widespread purpuric skin rash, coagulation defects, septic shock and death within a few hours of onset of symptoms[11]. Other presentations include a predominantly meningitic illness which may or may not be accompanied by a purpuric rash, primary meningococcal arthritis, pneumonia, conjunctivitis and, more rarely sinusitis, endocarditis and necrotising fasciitis[13-15]. Occasionally a more chronic picture may be encountered in association with positive blood cultures often with cutaneous lesions and arthritis.

N. meningitidis may also be isolated from the lower genital tract or rectum in men and women during screening for gonorrhoea and may be implicated in genital tract infections[16,17]. Rare deficiencies of the later stages of the complement and properdin pathway (or treatments that inhibit the complement pathway) can predispose to recurrent infections with uncommon *N. meningitidis* serogroups, non-groupable meningococci and *Neisseria*-related bacteria presenting as meningococcal disease[18]. Disseminated meningococcal infection, although rare, may also be found in patients who are infected with HIV[19].

Epidemiology

Meningococcal disease occurs worldwide. Thirteen serogroups have been identified (based on unique capsular polysaccharides), six of which cause the majority of infections (A, B, C, W135, X and Y)[20]. Serogroup B is the most prevalent serogroup in the UK followed by serogroup C[21,22]. Incidence of invasive serogroup Y in the UK has increased over recent years. Natural immunity in the population to group W135 and Y meningococci has been shown to be low across all age groups[23].

The incidence and case fatality are highest in infants less than one year of age in whom the signs of early infection may be more difficult to detect[8,21,22]. There is a second but lower peak of infection in the 15-24 year age group and a seasonal peak in the winter months[24].

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[1,2]

SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

1 Safety Considerations[1,2,25-39]

1.1 Specimen Collection, Transport and Storage[1,2,25-28]

Use aseptic technique.

Collect swabs into appropriate transport medium.

Transport swabs in transport medium in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[1,2,25-39]

N. meningitidis is a Hazard Group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2.

Due to the severity of the disease and the risks associated with generating aerosols of the organism, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols)[31].

N. meningitidis can cause severe and sometimes fatal disease. Laboratory acquired infections have been reported[40,41]. The organism infects primarily by the respiratory route. An effective vaccine is available for some meningococcal groups.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens

Oropharyngeal swabs, nasopharyngeal swabs

2.2 Optimal Time and Method of Collection[42]

For safety considerations refer to Section 1.1.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[43-47].

2.3 Adequate Quantity and Appropriate Number of Specimens[42]

N/A

3 Specimen Transport and Storage[1,2]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[42].

Specimens should be transported and processed as soon as possible[42].

Recovery of meningococci is compromised if culture is delayed[10].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[42].

Direct plating when the swab is taken should be considered.

4 Specimen Processing/Procedure[1,2]

4.1 Test Selection

N/A

4.2 Appearance

N/A

4.3 Sample Preparation

N/A

4.4 Microscopy

N/A

4.5 Culture and Investigation

Inoculate each plate with swab. (Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Screening for *N. meningitidis* case or contact	Swab	GC selective agar	35-37	5-10% CO_2	40-48hr	daily	*N. meningitidis*

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Neisseria species	species level ID 6 - Identification of *Neisseria* species

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved nation reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales
http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Refer *N. meningitidis* for confirmation of identification, typing and susceptibility testing.

5 Reporting Procedure

5.1 Microscopy

N/A

5.2 Culture

Negative

N. meningitidis not isolated.

Positive

N. meningitidis isolated and report serogroup if known or state "Further identification to follow".

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically when available.

Interim/final written report, 16 – 72hr stating, if appropriate, that a further report will be issued.

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[48,49] or Equivalent in the Devolved Administrations[50-53]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

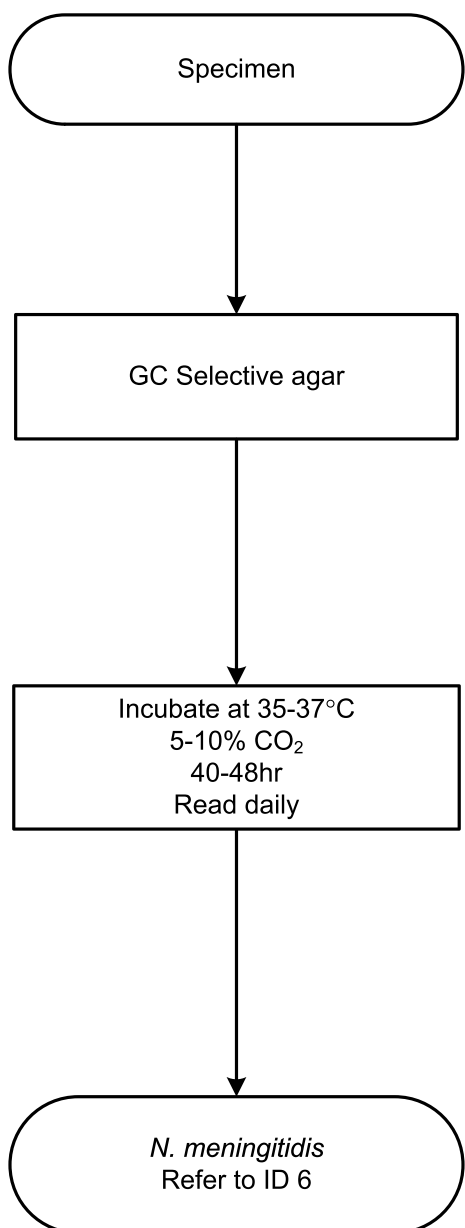
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAIs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/

Other arrangements exist in Scotland[50,51], Wales[52] and Northern Ireland[53].

Appendix: Screening for *Neisseria meningitidis*



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UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 51 Screening for *Neisseria meningitidis*

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st CONSULTATION 06.10.08 – 07.11.08

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	30/10/2008	**Lab Name**	NPHS, Cardiff Lab
Section	1.3		
Comment			
My only comment here is that the statement in Section 1.3, Specimen Processing " *N. meningitidis* is in Hazard Group 2 although in some cases the nature of the work may dictate full Containment Level 3 conditions" needs definition as I can only think of three possible scenarios when this may be likely:- a) if the MSC in a laboratory is housed at CL3; b) if the work involves the propagation of hugely increased numbers of the agent such as for vaccine production or c) if the agent is reclassified as Hazard Group 3.			
Recommended Action	**ACCEPT** This section has been strengthened in the SMI.		

Comment Number	2		
Date Received	22/10/2008	**Lab Name**	NPHS, Cardiff Lab
Section			
Comment			
This is very good. I note that as the title is screening for meningococci, the only plate recommended is a GC selective. As most of ours, and that's not many, are throat swabs from contacts, we add a GC selective to whatever plates are to be put up for that specimen type. Subsequently report a specific negative, eg N. meningo not isolated. Happy to say that this therefore meets with our current practice.			
Recommended Action	**NONE**		

2nd CONSULTATION 21.09.12 – 14.12.12

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	18/09/2012	**Lab Name**	MSTAG
Section	a. Introduction b. 1.3 c. 2.1		
Comment			
a. Nasopharyngeal swabs may be difficult to obtain.			

b. Comment regarding salivary samples is irrelevant to this method as swabs recommended. c. Use of word propagation in paragraph 2, should the wording be manipulation.	
Recommended Action	a. **ACCEPT** Text updated. b. **ACCEPT** Text removed. c. **PARTIAL ACCEPT** Safety considerations reviewed. Text updated to: *'N. meningitidis is a Hazard Group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2.* *Due to the severity of the disease and the risks associated with generating aerosols of the organism, any manipulation of suspected isolates of N. meningitidis should always be undertaken in a microbiological safety cabinet until N. meningitidis has been ruled out (as must any laboratory procedure giving rise to infectious aerosols).* *N. meningitidis can cause severe and sometimes fatal disease. Laboratory acquired infections have been reported. The organism infects primarily by the respiratory route. An effective vaccine is available for some meningococcal groups.'*

Comment Number	2		
Date Received	21/09/2012	**Lab Name**	Manchester Royal Infirmary
Section	2.1		
Comment			
'*N. meningitidis* is a Hazard group 2 organism; the processing of most diagnostic work can be carried out at Containment Level 2 unless infection with *N. meningitidis* is suspected.' The EP is directed at screening of *N. meningitidis* not routine work, and therefore the work should be carried out in a safety cabinet? For example what do we mean by unless infection with *N. meningitidis* is suspected these swabs are only being processed because we suspect them to be positive? Perhaps it could be replaced with: swabs from suspected cases should be processed in safety cabinet?			

Recommended Action	ACCEPT Safety considerations reviewed. Text updated. Refer to comment 1c.

Comment Number	3		
Date Received	13/11/2012	Lab Name	Vaccine Preventable Infections Programme Board
Section	a. Scope b. Scope (second paragraph) c. Introduction d. Carriage e. Carriage f. Spectrum of disease g. Page 9 h. Section 1.2.2 i. Section 2.1 j. Section 2.6 k. Section 3.3		

Comment

a. It has a very limited scope but within that scope it seems comprehensive.
b. Add '(the meningococcus)' after *Neisseria meningitidis*.
c. Change 'host nasopharynx flora' to 'nasopharyngeal flora'.
d. Change 'nasopharangeal musocsa' to 'nasopharangeal mucosa'.
e. Nasopharangeal changed to nasopharyngeal.
f. Porpedin corrected to properdin and add '(or treatments that inhibit the complement pathway)'.

   Needs to be mentioned as the use of Eculizumab is more widespread not just PNH patients but now aHUS.
g. Change 'National Group C meningococcal vaccination programme' to 'National sero group C meningococcal vaccination programme'.
h. "Recovery of meningococci may be compromised if culture is delayed" should be changed to "recovery of meningococci is compromised if culture is delayed".
i. "e.g. for the propogation of N. meningitidis" This needs very careful thinking as could be construed as simply subbing out from one plate to another which is simply a Cat 2 function. The only time we consider Cat 3 is for large scale broth cultures. Maybe we could comment further ?
j. Minimum level of identification in the laboratory table change *N. meningitidis* to *Neisseria.*
k. "Report susceptibilities as indicated" should be changed to "Report susceptibilities as clinically indicated".

Recommended	a. **NONE**

Action	b. **ACCEPT** Text updated. c. **ACCEPT** Text updated. d. **ACCEPT** Text updated to ‘mucosa’. e. **ACCEPT** Text updated. f. **ACCEPT** Text updated. g. **ACCEPT** Text updated. h. **ACCEPT** Text updated. i. **ACCEPT** Text updated to ‘*Neisseria* species’. j. **ACCEPT** Text updated (section 5.3 in current PHE template). k. **ACCEPT** Text updated.

UK Standards for Microbiology Investigations

Investigation of infectious causes of dyspepsia

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, Microbiology Services, PHE

Technical | B 55 | Issue no: 7 | Issue date: 03.10.19 | Page: 1 of 16

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UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

PHE publications gateway number: 2015574

UK Standards for Microbiology Investigations are produced in association with:

Contents

Acknowledgments **2**

Amendment table **4**

1. General information **5**

2. Scientific information **5**

3. Scope of document **5**

4. Background **5**

5. Safety considerations **6**

6. Diagnostic tests/investigation **6**

7. Antimicrobial susceptibility testing **12**

8. Referral to reference laboratories **12**

Appendix: Diagnostic algorithm of Gastric biopsies for *Helicobacter pylori* **14**

References **15**

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	10/03.10.19
Issue number discarded	6
Insert issue number	7
Anticipated next review date*	03.10.22
Section(s) involved	**Amendment**
Title.	Title changed from investigation of Helicobacter pylori gastric biopsies.
Whole document.	The whole document has been reformatted to a new more interactive and comprehensive template. All the background, technical, scientific and legal information has been removed and uploaded to our webpage and can be accessed from this document via hyperlink.
Whole document.	The document scope has been changed to include: Laboratory based stool antigen ELISA (SAT), *Helicobacter pylori* IgG ELISA, Culture and microscopy of gastric biopsy.

*Reviews can be extended up to five years subject to resources available.

1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

This UK SMI describes the diagnosis of *Helicobacter pylori* infection in the investigation of dyspepsia. This will cover:

- Laboratory based stool antigen ELISA (SAT)
- Serology*: Helicobacter pylori* IgG ELISA
- Microscopy and culture of gastric biopsy

Other procedures used for *H. pylori* testing such as the Urea Breath Test (UBT) will not be covered in this UK SMI.

4. Background

In 1984 Warren and Marshall first proposed the association of *H. pylori* with peptic ulcer disease, and since then it has become established as the most clinically important species of *Helicobacter*[1]. It is recognized as the main cause of peptic ulcer disease and a major risk factor for gastric cancer[2]. *H. pylori* infection is also an independent risk factor for the development of atrophic gastritis, gastric ulcer disease, gastric adenocarcinomas, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas[2]. The species establishes a chronic infection in the majority of infected people, represented by chronic gastritis. Prominent mucosal inflammation is often evident in the antrum (antrum-predominant gastritis), predisposing to hyperacidity and duodenal ulcer disease. Many patients infected with *H. pylori* have recurrent abdominal symptoms (non-ulcer dyspepsia) without ulcer disease, and there appears to be a clinical benefit in eradicating *H. pylori* in these patients[3]. Acute symptoms of gastritis and epigastric pain, nausea and vomiting may occur and usually subside, but hyperchlorhydria may persist for much longer.

The detection and diagnosis of *H. pylori* infections has been of great interest. Initially invasive techniques (for example, tissue biopsies) were used for diagnosis. However, with progress in the diagnostic field, (especially molecular biology) non-invasive techniques are now routinely used within the clinical laboratory for initial diagnosis of infection.

The National Institute of Clinical Excellence (NICE) and PHE guidelines on dyspepsia states that a 'test and treat' strategy should be employed for cases of dyspepsia and suspected gastric and duodenal ulcer that have not previously been investigated[3-6]. Recommended tests include the urea breath test (UBT) and stool antigen test (SAT)[3-6]. Blood serology is less predictive of current infection than the UBT or SAT. Serology test results are variable and these tests should not be used in the elderly,

children or post treatment[5,6]. Near-patients serology tests are not recommended[5]. SAT and IgG serology tests both have a negative predictive value NPV>95%[7].

Following a positive result for *H. pylori*, eradication therapy consisting of a seven-day course of a proton pump inhibitor (PPI) with amoxicillin and either clarithromycin or metronidazole is given. An alternative first line treatment regimen is required if the patient is allergic to penicillin; detailed information regarding first and second line treatment options can be found in NICE clinical guidance 184: Dyspepsia and gastro-oesophageal reflux disease[4]. *H. pylori* culture and sensitivities on gastric biopsies should be considered after the first treatment failure if an endoscopy is carried out. Following a second treatment failure, culture and sensitivity should be performed on all cases[8]. The Maastricht IV consensus report also recommends that culture and sensitivities are carried out in areas where resistance to clarithromycin is above 20%[8,9].

In the UK *H. pylori* is frequently resistant to metronidazole (22% to 88%). Clarithromycin resistance is less common in the general population (3% to 68%). Levofloxacin resistance occurs in about 17% of isolates and is due to the widespread use of fluoroquinolones. *H. pylori* are rarely resistant to amoxicillin, rifampicin and tetracycline (~3%). *H. pylori* can also be treated with rifabutin a similar drug to rifampicin, but with different susceptibilities (resistance is extremely rare <1%)[10].

5. Safety considerations

Containment Level 2.

6. Diagnostic tests/investigation

6.1 Laboratory based stool antigen ELISA (SAT)

Stool antigen tests using an ELISA provide a valuable aid in the diagnosis of an active *H. pylori* infection[11]. The test is easy to perform and has the advantage of being non-invasive. Two types of stool antigen test are available; a laboratory-based enzyme-linked immunosorbent assay (ELISA) method and rapid near patient (immunochromatographic) kits. Over recent years SAT ELISAs using monoclonal antibodies instead of polyclonal antibodies have been developed. These have high accuracy for both primary diagnosis and post treatment diagnosis[8,12-14]. Near-patient testing kits are less reliable[8,15]. Evidence-based studies suggest that ELISA SAT is the most cost-effective means of diagnosing *H. pylori* infection[16,17].

6.1.1 Specimen type

Stools or refer to manufacturer's guidelines.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage:

Fresh faecal sample should be collected into a stool sample collection container. Collect a minimum of 1-2 mL liquid stool sample or 1-2 g solid sample.

If specimen is not processed on the same day it can be stored at 2-8 °C.

6.1.3 Laboratory processes (analytical stage)

Follow manufacturer instructions for details on the specific protocol for this test.

6.1.4 Post-laboratory processes (reporting procedures)

Interpreting and reporting results

Report SAT results as:

Positive report:

H. pylori antigen detected suggesting current infection

Negative report:

H. pylori antigen not detected suggesting absence of infection

6.2 IgG ELISA

H. pylori infection is regarded as a chronic infection and therefore only IgG is considered when carrying out serological tests for diagnosis[8]. The favoured method is standard ELISA. Commercial tests show variable accuracy and ideally validated IgG serology should only be used in the following situations[8,9].

- following recent use of antimicrobial and antisecretory drugs
- where there is ulcer bleeding, atrophy or gastric malignancy

6.2.1 Specimen type

Clotted blood or refer to manufacturer's guidelines

6.2.2 Pre-laboratory processes

Specimen collection, transport and storage:

Specimens should be collected, transported and processed according to manufacturer's instructions or locally validated data.

6.2.3 Laboratory processes (analytical stage)

Follow manufacturer instructions or local guidelines for details on the specific protocol for this test.

6.2.4 Post-laboratory processes (reporting procedures)

Interpreting and reporting results

Report serology results as:

Positive report:

H. pylori IgG detected suggesting current or past infection

Negative report:

H. pylori IgG not detected suggesting absence of infection

6.3 Gastric biopsy

Gastric biopsy is the specimen of choice for the culture *of H. pylori*. Attempts to culture from other specimens have a low success rate.

Culture of the organism is the most specific method and offers opportunity for conventional antimicrobial susceptibility testing. This is important in predicting and evaluating the efficacy of treatment, and in identifying re-infections. With the adoption of the ‘test and treat’ strategy as recommended by NICE, the main rationale for obtaining a biopsy for culture is to establish the susceptibility of the isolate.

Organisms may be stained using Giemsa or Gram stains according to preference. Sensitivities of up to 90% have been reported if two biopsies are examined, but this method requires technical expertise[18].

6.3.1 Specimen type

Gastric biopsy

6.3.2 Pre-laboratory processes

Specimen collection, transport and storage:

Ideally biopsies should be taken before antimicrobial therapy is begun, however a ‘test and treat’ strategy for the diagnosis of *H. pylori* is recommended by NICE[19] and therefore most samples referred for culture will be due to treatment failure. A period of at least two weeks should have elapsed since the last dose of antimicrobial therapy before the collection of the specimen.

Gastric biopsy specimens are usually taken from the gastric antrum at endoscopy, and sometimes from the main body of the stomach depending on location of inflammation. Duodenal biopsies will be taken in cases with duodenal ulcers.

Specimens should be transported and processed as soon as possible (preferably within 6hr)[20,21].

If processing is delayed >6hr, refrigeration is recommended[20,22] .

If transported on ice, glycerol containing media should be used to avoid freezing[23].

It is important to maintain a moist atmosphere during transport.

Where culture is to be carried out within six hours[23-26] :

The biopsy should be placed in a small, CE marked, leak proof container such as a bijou bottle, containing a small amount (approximately 100µL) of sterile isotonic saline to prevent desiccation[26]. Alternatively, Dent’s transport medium can be used.

Note: Sensitivity of the microscopy may be reduced if the biopsy is submerged in the saline, because mucus globules form and production of a satisfactory smear becomes difficult.

Where delays of >6hr are expected[23-26]:

The biopsy should be covered with approximately 1mL brain heart infusion broth in a small sterile container, such as a bijou bottle, and stored at 4°C for up to 48hr. Alternatively Dent’s transport medium can be used.

Biopsies may be stored for up to 6 months at -70°C in broth containing 20-25% glycerol although viability will be significantly reduced.

6.3.3 Laboratory processes (analytical stage)

Culture

Sample preparation

For safety considerations refer to Section 2.

Cut the biopsy finely with a sterile scalpel.

Homogenisation can be performed if needed using a sterile homogenising system.

Specimen processing

Inoculate each agar plate with a swab containing the homogenised biopsy.

For the isolation of individual colonies, spread inoculum with a sterile loop.

Note: The simultaneous subculture of known control strains of *H. pylori* is recommended, especially if susceptibility testing is to be performed.

The following control strains may be used[10]:

- type strain – NCTC 11637
- Metronidazole and Clarithromycin sensitive strain – NCTC 12455
- Metronidazole and Clarithromycin resistant strain – NCTC 11637

Investigation

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmosphere	Time		
Gastritis	Gastric biopsy	Dent's selective agar or alternative *H. pylori* selective agar*	35-37	Microaerobic Moist chamber containing hydrogen (3-5%)	10 d	Every 48hr	*H. pylori*
		Blood agar 10% horse blood[27]	35-37	Microaerobic Moist chamber containing hydrogen (3-5%)	10 d	Every 48hr	
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Gastritis - Biopsy urease test if not already performed	Gastric biopsy	Christenson's Urea broth **	ambient	air	24hr	hourly up to 6hr and again at 24hr	*H. pylori*

in endoscopy suite							
* Alternative culture media can be used if approved by manufacturer or validated locally. ** Brain heart infusion (BHI) broth can be used to start the culture process[28].							

Identification

Refer to UK SMI ID 26 - Identification of *Helicobacter* species for organism identification.

Minimum level of identification in the laboratory

H. pylori	species level

Microscopy

Refer to TP 39 – Staining procedures.

Microscopy is carried out using carbol fuchsin or Sandiford's counter stain[29].

Pick up the biopsy (or piece of finely cut biopsy) with a sterile swab and smear vigorously on to a clean microscope slide (a sterile slide is required if microscopy is performed before culture).

Gram or Giemsa stains are suitable for immediate observation of the organism although Gram stain sensitivity is poor.

Other techniques for gastric biopsy examination

Techniques for examination of gastric biopsies taken at endoscopy include[21,30,31]

- PCR
- histology
- urease test

The order in which any or all of the tests are performed will be in accordance with local protocol.

PCR

PCR has been used extensively for the diagnosis of *H. pylori* form gastric biopsy specimens and saliva[9,31]. It is also used for detecting clarithromycin resistance. Mainly used as a research tool, PCR is valuable for collecting information on the presence of potential virulence markers in the strain, which might have implications for the development of severe disease or efficacy of eradication. PCR for *H. pylori* has not made its way to be a routine test as it is a technically demanding and expensive test compared to culture, histology and the rapid urease tests.

Histology

Histology examination is as sensitive as culture when detecting *H. pylori*, and has a high degree of specificity[30]. Currently Giemsa staining is most widely used, immunostaining may also be used and increases sensitivity and specificity[9].

Urease test

The urease test is often performed on biopsies in the endoscopy suite; therefore, only culture and microscopy is usually required in the laboratory. The urease test also known as the rapid urease test (RUT) or Campylobacter-like organism test (CLO test), is a rapid, sensitive and cost-effective test[9,31]. Positive results are often available within minutes but negative reporting may take a great deal longer, according to manufacturers' instructions. It is recommended for use in combination with either culture or histology, depending on local facilities. This test is often carried out in the endoscopy suite. Commercial kits are available which are highly accurate but also expensive.

6.3.4 Post-laboratory processes (reporting procedures)

Culture

Interpreting and reporting results

Report culture results as:

Positive report

H. pylori isolated

Negative report

H. pylori not isolated

Culture reporting time

- Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected.
- Urgent results should be telephoned or transmitted electronically in accordance with local policies.
- Final written or computer-generated reports should follow preliminary and verbal reports as soon as possible.
- Culture results are usually available within 10 days but may take up to 12 days (15 days if antimicrobial susceptibility testing is required)

Microscopy

Interpreting and reporting results

Report microscopy results as:

Gram stain

Report presence or absence of *H. pylori*-like organisms.

Microscopy reporting time

- All results should be issued to the requesting clinician as soon as they become available.
- Urgent results should be telephoned or transmitted electronically in accordance with local policies.

7. Antimicrobial susceptibility testing

Disc diffusion criteria for antimicrobial susceptibility testing of *H. pylori* have not been defined therefore an MIC method should be used[32].

If a commercial MIC method is used, manufacturer's instructions should be followed.

Refer to EUCAST guidelines for breakpoint information.

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

It is recommended that the antimicrobials in bold in the table below are reported.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
H. pylori	**Amoxicillin** **Clarithromycin** **Metronidazole** **Levofloxacin** **Tetracycline**	Rifampicin	

7.1 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

8. Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

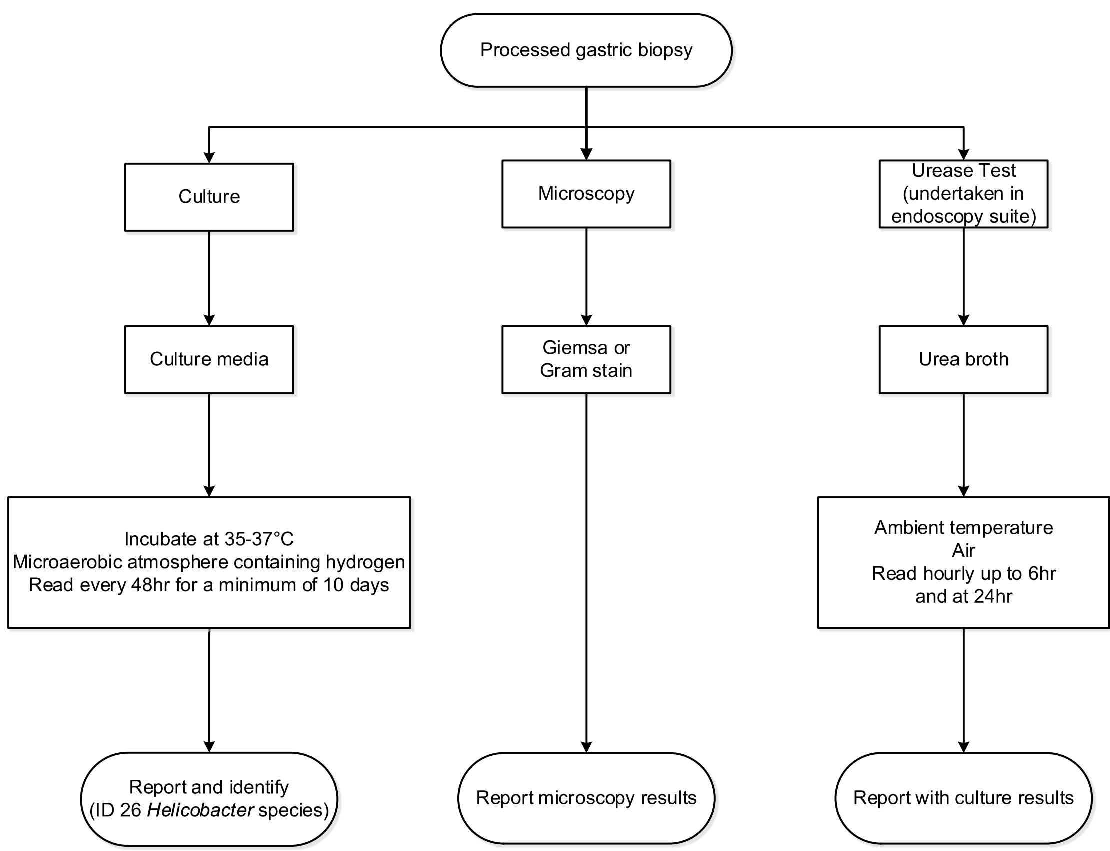
http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix: Diagnostic algorithm of Gastric biopsies for *Helicobacter pylori*



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UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

B 55 Investigation of infectious causes of dyspepsia

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

This publication was created by Public Health England (PHE) in partnership with the NHS.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 20/04/2018 – 04/05/2018

Version of document consulted on: B 55dc+

Proposal for changes

Comment number	1		
Date received	04/05/2018	**Lab name/Professional body**	Laboratory
Section	a. 2.2 Optimal time and method of collection b. 3.1 Optimal transport and storage conditions		
Comment			
a. Suggest a period of 4 weeks should have elapsed since the last dose of antimicrobial therapy and Proton Pump Inhibiter (PPI) before the collection of the specimen. Depending on location of inflammation it is recommended to perform multiple gastric biopsies (5-6), at least 2 from the antrum and 2 from the anterior and posterior corpus respectively. Duodenal biopsies will be taken in cases with duodenal ulcers. Larger volumes increase the yield. All microbiology specimens should be taken with sterile forceps before the histology specimens are taken to reduce risk of contamination. b. Suggest addition: Where culture is to be carried out by the Reference Laboratory Gastric biopsies should be sent without delay, preferably within 24 hours of collection and ideally using specialised H. pylori transport medium (e.g. Dent's transport medium or equivalent). Alternatively, biopsies can be sent in sterile saline. Where there is greater than 24 hours delay in sending biopsies to the Reference Laboratory, store refrigerated and avoid allowing specimens to dry. Refer to PHE Bacteriology Reference Department User Manual for further details. (https://www.gov.uk/government/publications/bacteriology-reference-department-brd-user-manual)			
Evidence			
Not completed.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	a. **NONE** This part of the document was removed as it does not form part of the scope. b. **PARTIALLY ACCEPT** Document has been updated to include information on transport of sample if culture is to be carried out before		

	or after 6 hours and not 24 hours. Storage at 4C has been suggested for up to 48 hours.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 4			
Date received	20/04/2018	**Lab name/Professional body**	Laboratory
Health benefits			
Not completed.			
Date received	26/04/2018	**Lab name/Professional body**	Laboratory
Health benefits			
Not aware of any issues.			
Date received	04/05/2018	**Lab name/Professional body**	Professional body
Health benefits			
Not completed.			
Date received	08/05/2018	**Lab name/Professional body**	Professional body
Health benefits			
Not completed.			

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Investigation of bronchoalveolar lavage, sputum and associated specimens

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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 57 | Issue no: 3.5 | Issue date: 08.05.19 | Page: 1 of 38

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Logos correct at time of publishing.

Contents

ACKNOWLEDGMENTS 2

AMENDMENT TABLE 4

UK SMI: SCOPE AND PURPOSE 7

SCOPE OF DOCUMENT 9

INTRODUCTION 9

TECHNICAL INFORMATION/LIMITATIONS 16

1 SAFETY CONSIDERATIONS 19

2 SPECIMEN COLLECTION 19

3 SPECIMEN TRANSPORT AND STORAGE 20

4 SPECIMEN PROCESSING/PROCEDURE 21

5 REPORTING PROCEDURE 28

6 NOTIFICATION TO PHE, OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS 29

APPENDIX 1: BAL SPECIMENS FOR CULTURE 31

APPENDIX 2: SPUTUM SPECIMENS FOR CULTURE 32

REFERENCES 33

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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	13/08.05.19
Issue no. discarded.	3.4
Insert issue no.	3.5
Section(s) involved	**Amendment**
Appendix 1.	Incubation time for Sabouraud plate changed from 40 hours to 5 days.

Amendment no/date.	12/01.03.18
Issue no. discarded.	3.3
Insert issue no.	3.4
Section(s) involved	**Amendment**
Appendix 2.	“Pneumonia or flu like symptoms” replaced by “*legionella* suspected” to be in agreement with sputum table.

Amendment no/date.	11/24.08.17
Issue no. discarded.	3.2
Insert issue no.	3.3
Section(s) involved	**Amendment**
Appendix 1.	Incubation time for *B. cepacia* has been clarified.

Amendment no/date.	10/28.12.16
Issue no. discarded.	3.1
Insert issue no.	3.2
Section(s) involved	**Amendment**

Introduction.	Subheading on *Nocardia* and *Actinomyces* infections has been updated to include bronchoalveolar lavage.
Safety Considerations.	Section has been strengthened with additional information on handling specimens suspected to be Containment level 3 organisms.
Specimen processing/procedure.	Section 4.4.2 Supplementary • For Sputum, the appropriate staining technique for fungi has been updated. Sections 4.5.3 and 4.5.4 have been updated with information on the incubation atmosphere and time for the *Legionella* species. Footnotes have been added for clarity.
Appendix 1 and 2.	Flowcharts amended to reflect information on section 4.5.3.

Amendment no/date.	9/09.12.15
Issue no. discarded.	3
Insert issue no.	3.1
Section(s) involved	**Amendment**
Appendix 2.	Error in flowchart amended.

Amendment no/date.	8/02.10.15
Issue no. discarded.	2.5
Insert issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope.	Cross links to other UK SMIs improved.
Introduction.	Cystic fibrosis section reviewed and expanded. Information on Legionella included. The section on fungal infections reviewed and updated. Section on types of specimen reviewed and

	updated. Deliberate release organisms added to the introduction. Interpretation of gram stains section expanded.
Safety considerations.	Additional information on Hazard Group 3 Fungi inserted in to the document.
Culture and investigation.	Information for BAL expanded to clarify the semi-quantitative method. Information on Legionella culture inserted. Fungal section has been divided in to cystic fibrosis and non-cystic fibrosis patients. A section on molecular methods has been inserted. Culture for *M. abscessus* added to the cystic fibrosis section.
References.	References reviewed and updated.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratorieshttp://www.hpa-standardmethods.org.uk/. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Bronchial aspirate, transthoracic aspirate, bronchoalveolar lavage, transtracheal aspirate, bronchial brushings, protected catheter specimens, bronchial washings, endotracheal tube specimens, sputum – expectorated

This SMI describes the isolation of organisms known to cause bacterial and fungal respiratory infection from sputum, bronchoalveolar lavage and associated specimens. Different tests are carried out on different sample types depending on the patient group. For information on *Bordetella pertussis* and *Bordetella papapertussis* see B 6. For Investigation of specimens for *Mycobacterium* species see B 40. For viruses see S 2 – Pneumonia, and G 8 - Respiratory viruses.

This SMI should be used in conjunction with other SMIs.

Introduction

Recovery and recognition of organisms responsible for pneumonia depends on:

- the adequacy of the lower respiratory tract specimen
- avoidance of contamination by upper respiratory tract flora
- the use of microscopic techniques and culture methods
- current and recent antimicrobial treatment

The expression lower respiratory tract infection (LRTI) includes pneumonia, where there is inflammation of the lung parenchyma, and infections such as bronchiolitis that affect the small airways. Lung abscess, where the lung parenchyma is replaced by pus filled cavities, and empyema, where pus occupies the pleural space, are less common manifestations of LRTI.

Distinction between tracheobronchial colonisation and true pulmonary infection can prove difficult.

Pneumonia

Pneumonia can be classified according to whether it is community acquired or nosocomial (often defined as presenting more than 48 hours after hospitalisation). It may be primary, occurring in a person without previously identified risk factors, or secondary. Many conditions are associated with an increased risk of pneumonia. Common risk factors include chronic lung diseases such as chronic obstructive pulmonary disease (COPD), diabetes mellitus, cardiac or renal failure and immunosuppression (either congenital or acquired). Reduced level of consciousness and weakness of the gag and cough reflexes are risk factors for aspiration pneumonia. Recent infection with respiratory viruses, particularly influenza, is also a risk factor. There are clinical signs and laboratory indices that can be used to assess the severity of pneumonia in an individual patient, some of which are predictive of an increased risk of death if present[1].

The aetiology of pneumonia varies according to whether it has been acquired in the community or in hospital and the risk factors present. Many of the bacteria found as colonisers of the upper respiratory tract have been implicated in pneumonia. Antibiotic

treatment and hospitalisation affect the colonising flora, leading to an increase in numbers of aerobic Gram negative bacilli[2]. These factors affect the sensitivity and specificity of sputum culture as a diagnostic test and results must always be interpreted in the light of the clinical information[3]. Sputum culture results are often unreliable and sensitivity of culture is poor for many pathogens, although culture and antibiotic sensitivities may be of value in sputum specimens from patients with severe exacerbation of COPD[4].

Community acquired pneumonia[5]

The commonest cause of community acquired pneumonia is *Streptococcus pneumoniae*, which is responsible for up to 60% of cases in community based surveys and may be multi-drug resistant. It can affect individuals of any age, including those without known risk factors. Other bacterial pathogens tend to cause pneumonia in the presence of specific risk factors. Patients with COPD and patients infected with HIV are additionally at risk of pneumonia caused by *Haemophilus influenzae* and *Moraxella catarrhalis*. *Staphylococcus aureus* pneumonia occurs either in the context of recent influenza infection or, less commonly, as a result of blood borne spread from a distant focus, COPD or aspiration. Aerobic Gram negative rods are rare causes of community acquired pneumonia. Occasionally, *Klebsiella pneumoniae* causes severe necrotising pneumonia, typically in patients with a history of alcohol abuse and homelessness (“Friedländer’s pneumonia”).

A number of other pathogens cause atypical pneumonia within the community[6]. *Mycoplasma pneumoniae* causes up to 20% of community acquired pneumonia, second only to *S. pneumoniae.* Infection with *Mycoplasma pneumoniae* tends to occur in epidemics every 4-5 years and affects younger age groups. *Chlamydophila pneumoniae* is an exclusively human pathogen. Pneumonia in a minority of individuals is caused by *Chlamydophila psittaci* and *Coxiella burnetii* and occurs in individuals with a relevant exposure history (birds and farm animals). *Legionella pneumophila* is a rare cause of outbreaks of community acquired pneumonia usually where there is a recent history of travel. Respiratory viruses, such as respiratory syncytial virus (RSV), influenza and adenoviruses may commonly cause primary viral pneumonia (see G 8 - Respiratory viruses)[7].

Hospital acquired pneumonia[8]

Hospital acquired pneumonia is the second commonest type of nosocomial infection. Risk is increased by the presence of underlying disease and by various interventions and procedures[9]. Mechanical ventilation is a major risk factor. Patients with critical illnesses requiring prolonged mechanical ventilation are susceptible to multi-resistant *Pseudomonas aeruginosa* and *Acinetobacter* species (eg *Acinetobacter baumannii*). Aerobic Gram negative bacilli, including members of the Enterobacteriaceae (such as *Klebsiella* and *Enterobacter* species) and *P. aeruginosa* are implicated in up to 60% of cases[10]. Intravascular catheters and nasal carriage are risk factors for pneumonia caused by meticillin resistant *S. aureus* (MRSA). *Legionella* species are also an occasional cause of hospital-acquired pneumonia.

Aspiration pneumonia

Aspiration pneumonia occurs when oropharyngeal contents are introduced into the lower respiratory tract. Reduced level of consciousness, for instance following head injury or drug overdose is a risk factor, as are weak gag and cough reflexes which can follow a stroke or other neurological disease.

Lung abscess

Lung abscess may develop secondary to aspiration pneumonia, in which case the right middle zone is most frequently affected. Other organisms may give rise to multifocal abscess formation and the presence of multiple small abscesses (<2cm diameter) is sometimes referred to as necrotising pneumonia. Pneumonia caused by *S. aureus* and *K. pneumoniae* may show this picture. Nocardiosis, almost always occurring in a setting of immunosuppression, may present as pulmonary abscesses. Abscesses as a result of blood borne spread of infection from a distant focus may occur in conditions such as infective endocarditis.

Burkholderia pseudomallei may cause lung abscesses or necrotising pneumonia in those who have visited endemic areas (mainly south east Asia and northern Australia) especially in the presence of diabetes mellitus[11].

Lemierre's syndrome or necrobacillosis originates as an acute oropharyngeal infection. Infective thrombophlebitis of the internal jugular vein can lead to septic embolisation and metastatic infection. The lung is most frequently involved and multifocal abscesses may develop. *Fusobacterium necrophorum* is the most common pathogen isolated from blood cultures in patients with this syndrome[12].

Cystic fibrosis[13]

Cystic fibrosis (CF) is caused by a defect in the CF transmembrane conductance regulator gene that affects the transport of ions and water across the epithelium[14]. This leads to progressive pulmonary disease associated with pulmonary infections, which are the major cause of morbidity and mortality in CF patients. The major pathogens are *S. aureus*, *H. influenzae* (usually non-encapsulated in CF patients), *S. pneumoniae* and pseudomonads, particularly mucoid *P. aeruginosa* strains[14,15]. Strains of *P. aeruginosa* with differing antibiotic susceptibilities may be isolated from a single sample. Anaerobes may also be present, together with *Aspergillus* species and mycobacteria other than *Mycobacterium tuberculosis* (MOTT)[16].

Nucleotide analysis of *rec*A gene sequences suggests that *Burkholderia cepacia* complex consists of several closely related genomovars[17]. Transmission of *B. cepacia* complex between patients may occur and some patients succumb to "*B. cepacia* syndrome" which is a rapidly fulminating pneumonia sometimes accompanied by septicaemia[18]. Subsequent to early reports more species have been included within this title and the patients prognosis is poor[13,19,20].

Non-tuberculosis Mycobacteria are an increasing problem for this patient group; *M. abscessus* more so than the others[21]. Testing should be considered in patients who show deteriorating lung function where no clear pathogen has been identified[22-25].

Resistance to antibiotics, particularly in *Burkholderia* spp., *Stenotrophomonas maltophilia* and *P. aeruginosa*, limits the options for treatment[26].

Organisms such as *Ralstonia*, *Achromobacter* and *Pandoraea* are emerging pathogens in chronic structural lung disease. Viruses have also been implicated[27,28].

For more information on this area refer to “Laboratory standards for processing Microbiological Sample from People with Cystic Fibrosis”[29].

Mycobacterial disease

Primary pulmonary infection with *Mycobacterium tuberculosis* may lead to the formation of the ‘primary complex’, particularly in childhood. The pulmonary focus may

be relatively small, but the draining hilar lymph nodes become greatly enlarged and may rupture, spreading infectious material into other areas of the lung. It is at this stage that miliary spread to other organs may occur via blood and lymphatics. Adolescents and adults may have asymptomatic primary infection, a typical primary complex or infection which progresses to typical chronic cavitating tuberculosis. Chronic cavitating disease is usually seen in reactivated primary infection and the lung apices are most commonly involved. The cough that accompanies this process produces aerosols of infectious particles, which is the route by which other persons may become infected. Mycobacteria other than tubercle bacilli have been recognised as causing human disease, particularly in those with immunosuppression or underlying disease. These include *Mycobacterium avium-intracellulare*, *Mycobacterium abscessus*, *Mycobacterium kansasi*, *Mycobacterium malmoense, Mycobacterium xenopi*, *Mycobacterium fortuitum* and *Mycobacterium haemophilum*. They are often resistant to standard antituberculous chemotherapy. Refer to B 40 - Investigation of specimens for *Mycobacterium* species.

Legionella disease

Transmission is by inhalation of an aerosol of the organism, either from an environmental source or occasionally iatrogenically following a respiratory tract manipulation such as humidification or nebulisation of infected material.

Pneumonia is the most common manifestation of *Legionella* infections. Severity varies from mild to severe, life–threatening disease. Onset is usually abrupt with pyrexia, myalgia, headache and non-productive cough following, commonly, a 2-10 day incubation period. The incubation time has been found to be as long as 20 days in some cases involving whirlpool baths and spas[30]. Watery diarrhoea may be present and neurological symptoms ranging from mild headache to encephalopathy may also occur[31]. Chest X-rays show pulmonary infiltrates progressing to consolidation often with pleural effusion[32].

Pontiac fever/non-pneumonic disease is an acute febrile illness occurring 24 – 48 hours after exposure to any species, but particularly to *L. pneumophila*, *Legionella feeleii*, *Legionella micdadei* and *Legionella anisa*[33-36]. Superficially, the disease resembles influenza and is usually self-limiting, without pneumonic involvement. It has been found that children have a shorter incubation period than adults and display symptoms such as ear ache and rashes, whereas common symptoms in adults included fever, dizziness, headaches, fatigue, arthralgia and abdominal pain[37].

Nocardia and *Actinomyces* infections[38,39]

Nocardiosis and actinomycosis are rare conditions that may affect other systems apart from the lungs.

Nocardia species are most often seen in the lung where they cause acute, often necrotising pneumonia. This is commonly associated with cavitation. It may also produce a slowly enlarging pulmonary nodule and pneumonia that is often associated with empyema. Immune defects associated with alcoholism, organ transplantation and HIV infection are present in the majority (60% plus) of patients presenting with nocardiosis.

Actinomyces species cause a thoracic infection that may involve the lungs, pleura, mediastinum or chest wall. Cases often go unrecognised until empyema or a chest wall fistula develops. Aspiration of oral contents is a risk factor for the development of

thoracic actinomycosis, thus predisposing conditions include alcoholism, cerebral infarction, drug overdose, general anaesthesia, seizure, diabetic coma or shock.

The appropriate specimens for investigation of both these organisms are pus, tissue and biopsy (which include bronchoalveolar lavage) samples (see B 14 - Investigation of abscesses and deep-seated wound infections and B 17 - Investigation of tissues and biopsies).

Parasitic infections[40]

Several helminth infections may give rise to the syndrome Tropical Pulmonary Eosinophilia, characterised by patchy pulmonary infiltrates and eosinophilia accompanied by symptoms of cough, fever and weight loss. These signs and symptoms are associated with passage of larval forms through the lungs and include *Ascaris lumbricoides*, hookworms and *Strongyloides stercoralis*.

The lung fluke, *Paragonimus westermanii* has a wide distribution and is particularly prevalent in the Far East, Indian subcontinent and West Africa. Human infection is acquired by consumption of uncooked freshwater crabs or crayfish that harbour encysted metacercariae. Although infection may be asymptomatic, heavy infestations are manifested by pulmonary infiltrates as above which may progress to chronic productive cough with pleuritic chest pain. Ova of *P. westermanii* are demonstrable in sputum (See B 31 - Investigation of specimens other than blood for parasites).

Fungal infections[41]

Candida species are extremely rare causes of LRTI. Occasionally infection occurs as a result of haematogenous seeding. Diagnosis is difficult given that the airways may become colonised in compromised patients treated with antibiotics.

Invasive aspergillosis still remains a life threatening infection in patients severely immunocompromised and contributes to the morbidity in cancer patients[42]. Underlying risk factors include patients receiving corticosteroids, individuals with haematological malignancies and those with previous pulmonary infections.

Aspergillus fumigatus species complex is one of the most prevalent species to cause fungal infections and a significant number of cases go undiagnosed, owing to the lack of sensitivity of tests available. Screening patients susceptible to fungal infections for the antigen galactomannan in serum and BAL in conjunction with molecular detection methods (eg 18SrRNA, ITS region) increase the diagnosis[43,44]. However, detection of fungal DNA cannot determine colonisation from active infection[45].

Pneumocystis pneumonia is caused by *Pneumocystis jirovecii*. It is the commonest cause of severe pneumonia in patients with advanced HIV infection, and is considered an AIDS defining illness[46]. Pneumocystis pneumonia also occurs in numerous other immunocompromised adults and children. It presents sub-acutely with cough, fever and hypoxia as the cardinal features, and is often subtle initially. The best diagnostic specimens are a BAL and transbronchial biopsies, but obtaining the latter carries some risk to the patient. BAL and induced sputum and mouthwash specimens are useful for molecular detection methods[47-49].

Some rare fungal causative agents of LRTI are endemic to defined geographical areas. Although many infections are subclinical, clinically apparent infections are occasionally imported into the UK. These illnesses occur in immunocompetent individuals and are reported to be more severe in patients who are immunocompromised. The diagnosis should be considered in travellers returning from

endemic areas who present with respiratory illness or pneumonia, particularly if they fail to respond to standard therapy. These infections include: histoplasmosis, caused by *Histoplasma capsulatum* (south east USA, Central America, Africa, Australia and eastern Asia); Coccidioidomycosis, caused *by Coccidioide immitis* and *C. posadasii* (south west USA, Central and South America) and blastomycosis caused by *Blastomyces dermatitidis* (eastern USA, Central and South America and Africa). These infections do present with distinguishing characteristics, however it is often difficult to differentiate them clinically from other causes of respiratory infection, particularly in their early stages. Paracoccidioidomycosis caused by *Paracoccidioides brasiliensis* (Central and South America) usually causes asymptomatic primary pulmonary infection. *Talaromyces* (previously *Penicillium*) *marneffei* (South east Asia, southern China) should also be considered when the travel history supports it. Fungal infections may reactivate if immune function declines.

Cryptococcosis is an unusual cause of pneumonia, usually in the immunocompromised host, and may be associated with meningitis, and is an AIDS defining illness. Pneumonia can be caused by *Cryptococcus neoformans.* This pathogen has worldwide distribution. Detection of the circulating cryptococcal antigen in the serum or BAL fluid is consistent with the diagnosis of cryptococcal pneumonia.

Types of specimen[9]

Expectorated sputum samples

Sputum samples are known to have issues with contamination. Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. Ventilator associated pneumonia carries a high mortality but is difficult to diagnose clinically and microbiologically. The criteria for diagnosis remain controversial. The poor sensitivity and specificity of sputum culture in the diagnosis of pneumonia in hospital ventilated patients has led to the development of a variety of techniques for obtaining lower respiratory tract specimens some involving the use of fibreoptic bronchoscopy.

Bronchoalveolar lavage (BAL)

A segment of lung is ‘washed’ with sterile saline after insertion of a flexible bronchoscope, thereby allowing recovery of both cellular and non-cellular components of the epithelial surface of the lower respiratory tract[50]. It is a reliable method for making a definitive aetiological diagnosis of pneumonia and other pulmonary infections[51,52].

Brush specimen results and bronchoalveolar lavage results are considered comparable by some authorities if a cut off of 10^4cfu/mL is used for the bronchoalveolar lavage although this is not recommended in this SMI because it remains controversial[53].

Non-directed bronchoalveolar lavage (NBL)

Non-directed techniques have been found to give results comparable to bronchoscopic methods[53-55]. A suction catheter, preferably a protected BAL catheter to minimise contamination, is passed down the endotracheal tube until resistance is met. An aliquot of sterile saline is injected and then aspirated. This method provides a lower respiratory tract sample without the need for bronchoscopy and without the attendant risks of transtracheal aspiration.

Bronchial aspirate

Bronchial aspirates are collected by direct aspiration of material from the large airways of the respiratory tract by means of a flexible bronchoscope.

Bronchial brushing

The technique of bronchial brushing uses a protected brush catheter in the bronchoscope (a brush within two catheters sealed at the end with a polyethylene glycol plug) to tease material from the airways. A pure bacterial count of greater than 10^3cfu/mL in a brush specimen obtained bronchoscopically has been found to correlate with a histological diagnosis of pneumonia[52].

Bronchial washings

Bronchial washings are collected in a similar fashion to bronchial aspirates, but the procedure involves the aspiration of small amounts of instilled saline from the large airways of the respiratory tract[50].

Protected catheter specimens

Material is collected from the lung via a bronchoscope in a similar way to bronchial brushing. An inner and outer catheter is used with a polyethylene glycol plug at the end to prevent contamination from the nasopharynx. When resistance is met the plug is expelled and the sample taken via the inner catheter.

Transthoracic aspirate

Samples of transthoracic aspirates are obtained through the chest wall via a needle passed between the ribs. This procedure may be undertaken to sample, for instance, an aspergilloma, abscess or any focal lung lesion that is accessible.

Transtracheal aspiration

Transtracheal aspiration is a procedure that carries clinical risks and is therefore rarely performed in the UK.

Tracheal aspirate

Tracheal aspirates are collected via the endotracheal tube. They are subject to the same limitations as sputum specimens.

Unusual organisms likely to be involved in a deliberate or accidental release of infection (bioterrorism or biological warfare)

In the absence of any other risk factor (eg foreign travel, clinical laboratory or veterinary work posing an infection hazard) cases or clusters of the organisms below could suggest the possibility of a deliberate or accidental release of micro-organisms. Such events require a rapid response; suspicion of deliberate or accidental release of micro-organisms must be notified urgently to the Public Health England 24hr Duty Doctor at Microbiology Services Colindale. Other arrangements exist in Scotland[56,57], Wales[58] and Northern Ireland[59].

If the following organisms are suspected, investigation should be carried out at containment level 3 unless otherwise stated. Suspect isolates should be sent to the appropriate reference laboratory for characterisation:

- *Bacillus anthracis* (Anthrax)
- *Brucella* species (Brucella)

- *Francisella tularensis* (Tularemia)
- *Burkholderia mallei* (Glanders)
- *Burkholderia pseudomallei* (Melioidosis)
- *Clostridium botulinum* (Botulism) may be investigated at Containment Level 2 in a Microbiological Safety Cabinet

Refer to ID 8 - Identification of *Clostridium* species

- *Coxiella burnetii* (Q fever)
- *Yersinia pestis* (Plague)

Note: *Brucella* species, *B. mallei, B. pseudomallei* and *Y. pestis* are listed in the databases of a number of commercially available kit-based identification systems; results should however be interpreted with caution.

Note: *B. anthracis, Brucella* species, *C. botulinum* and *Y. pestis* all cause disease which is reportable to the Local Authority Proper Officer under the Health Protection (Notification) Regulations 2010. A comprehensive list of diseases notifiable to the Local Authority Proper Office under the Health Protection (Notification) Regulations 2010 is available at:

https://www.gov.uk/notifiable-diseases-and-causative-organisms-how-to-report

Note: Brucellosis is reportable under the Zoonosis Order 1989.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[60,61]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Culture media

NAD-supplemented blood agar is inferior to combined use of blood and chocolate agars for isolation of *H. influenzae* and *S. pneumoniae*[62]. Slight improvement in isolation rates was demonstrated with prolonged incubation (48hr) of cultures.

Evaluations have shown that chocolate agar with bacitracin incorporated (or chocolate agar with a bacitracin disc) may be used in place of chocolate agar. Isolation rates of *H. influenzae* are not significantly different when this medium is used. Competing flora, however, are significantly reduced on bacitracin-incorporated agar and the quantity of growth of *H. influenza* is greater, which eases follow-up picking of colonies[63].

If chocolate agar with bacitracin incorporated into the agar is used then blood agar incubated in 5-10% CO_2 must be included for the isolation of *M. catarrhalis* and *S. pneumoniae*[63]. It may be difficult to differentiate between specific morphology of Streptococci on chocolate agar and in these instances a blood agar may be considered.

Burkholderia cepacia selective agar is recommended for use in the culture of specimens from patients with cystic fibrosis. It selectively supports the growth of *Burkholderia cepacia* and in this aspect is superior to CLED agar. *B. cepacia* selective agar may also grow *Burkholderia gladioli* and other pseudomonads.

BMPAα is recommended for clinical specimens, although there have been reports of cefamandole being inhibitory to some *Legionella* species[64-66]. Vancomycin sensitive strains have also been detected.

Incubation in 2-5% CO_2 can enhance growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*. This low level of CO_2 will not affect the growth of *L. pneumophila*, but CO_2 levels higher than 5% may inhibit growth[64].

All bacterial media are considerably inferior to fungal media, such as Sabouraud dextrose agar, for the detection of fungi. At risk patients should have specimens plated on fungal media routinely. Incubation temperature influences recovery: specimens with high loads of *Candida* species can obscure the growth of *Aspergillus* species, and culture at 42-45°C prevents *Candida* species growth, allowing *Aspergillus* species to grow. Refrigeration of specimens reduces the yield of mucoraceous moulds.

Interpretation of Gram stained smears

Gram stains on sputum specimens may be used for determining the quality of the specimen and for predicting likely pathogens by their characteristic appearance[67,68]. Determining the quality of the specimen is based on the numbers of polymorphonuclear leucocytes and squamous epithelial cells (SECs) present: purulent specimens may be selected for culture and non-purulent specimens or specimens contaminated with squamous epithelial cells may be rejected.

Sputum specimens are often not evaluated before culture, and preparation of slides for Gram staining occurs in parallel with specimen processing. Care must be taken in interpreting a Gram stained sputum smear as the use of antimicrobials may render organisms, which are visible in the smear, non-viable[68]. It may not be appropriate to identify organisms if gross contamination with oropharyngeal flora is evident for both BAL and sputum samples. The sensitivity of Gram stain can vary and is generally low and is often dependent on the individual reviewing the slide[40,68,69]. Gram staining may identify yeasts or hyphae, but are inferior to potassium hydroxide (KOH) and fluorescent brighteners.

Various methods of interpreting Gram stained smears by white blood cell and organism counts have been proposed. In BAL specimens Gram staining may be useful to predict results of quantitative culture[69]. In some cases, antimicrobial chemotherapy may be initiated on the results of the Gram stained smear before culture results are available.

Heat treatment of *Legionella* species

Some laboratories heat treat specimens when looking for *legionella* species. Although the method works well in certain contexts it has been shown to add very little to the clinical setting and is not included in this document[66,70,71].

1 Safety considerations[60,61,72-86]

1.1 Specimen collection, transport and storage[60,61,72-75]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[60,61,72-86]

Processing of diagnostic samples that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under appropriate containment conditions as determined by risk assessment, as required by Biological agents: managing the risks in laboratories and healthcare premises. This will normally be under full CL3 conditions.

For other sample types as a minimum it is recommended that the processing of all samples, including respiratory samples, which may result in generation of aerosols should be processed in a microbiological safety cabinet in CL2 conditions with additional precautions to minimise risk of aerosols production in accordance with the relevant risk assessment, ACDP and HSE guidelines.

Prior to staining for mycobacteria, the smeared material should be fixed by placing the slide on an electric hotplate (65 to 75°C), inside the safety cabinet, until dry and then placed in a rack or other suitable holder.

Note: Heat-fixing may not kill all *Mycobacterium* species[87]. Slides should be handled carefully.

Any mould isolated from patients with a travel history to areas where dimorphic or other Hazard Group 3 fungi are endemic should be processed at Category Level 3 until a hazard group 3 fungus is excluded.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must be placed in a suitable holder.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Bronchial aspirate, transthoracic aspirate, bronchoalveolar lavage, transtracheal aspirate, bronchial brushings, protected catheter specimens, bronchial washings, endotracheal tube specimens, sputum – expectorated

2.2 Optimal time and method of collection[88]

For safety considerations refer to Section 1.1.

Where possible all specimens should be fresh and taken before antimicrobial treatment is started[88].

Early morning freshly expectorated sputum is recommended for *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species).

Culture for *Legionella* species may still be successful after antimicrobial therapy has been started (ID 18 - Identification of *Legionella* species).

For sputum specimens the material required is from the lower respiratory tract, expectorated by deep coughing. When the cough is dry, physiotherapy, postural drainage or inhalation of an aerosol before expectoration may be helpful. Saliva and pernasal secretions are not suitable.

Early morning specimens for examination of *Mycobacterium* species should ideally be collected on at least 3 consecutive days (see B 40 - Investigation of specimens for *Mycobacterium* species). BAL and associated specimens need specialist collection according to local protocols.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium[89-93].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens[88]

Sputum - Ideally, a minimum volume of 1mL.

BAL - It is difficult to be specific on volume required; in principle, as large a volume as possible is preferred.

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

Note: Consideration should be given to use of chain of evidence forms in view of the potential for legal action in the event of infection with *Legionella* species[94].

3 Specimen transport and storage[60,61]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[88].

BAL and sputum should be processed promptly to give the best opportunity to culture pathogenic organisms and reduce the risk of overgrowth with contaminants. If processing has to be delayed up to 24 hours, refrigeration is preferable to storage at ambient temperature. If specimens are not processed on the same day that they are collected, this should be noted on the report and interpretation of results should be made with care[88,95,96].

4 Specimen processing/procedure[60,61]

4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and investigation of parasites (B 31 - Investigation of specimens other than blood for parasites) depending on clinical details.

Additional comments for sputum

Induced sputum may be sent for investigation for *P. jirovecii*.

Additional comments for BAL

Culture for *Mycobacterium* species should be performed on all BAL specimens unless special local arrangements do not require this.

Patients considered to be at risk of pulmonary aspergillosis, or in whom fungal infection is suspected, should have a portion of BAL fluid tested for Aspergillus galactomannan.

4.2 Appearance

Sputum

Specimens should not be rejected solely on macroscopic appearance. They may be described using the following terms: salivary, mucosalivary, mucoid, mucopurulent, purulent and/or bloodstained.

BAL

N/A

4.3 Sample preparation

Sputum

Follow manufacturer's instructions for the addition of 0.1% solution of dithiothreitol or N-acetyl L-cysteine (NALC) to sputum.

Dilute 10µL of homogenised sputum in 5mL of sterile distilled water.

Note: For mucoid samples treat as sputum.

BAL[40,97,98]

Centrifuge BAL at 1200 xg for 10 mins.

Tip off all but 0.5mL of supernatant and re-suspend centrifuged deposit in remaining fluid.

4.4 Microscopy

4.4.1 Standard

BAL

Mucoid specimens

Using a sterile loop select the most purulent or blood-stained portion of specimen and make a thin smear on a clean microscope slide for Gram staining.

Non-mucoid specimens

Using a sterile pipette place one drop of centrifuged specimen (see Section 4.3) on a clean microscope slide.

Spread this with a sterile loop to make a thin smear for Gram staining.

4.4.2 Supplementary

Sputum

Gram stain

Refer to TP 39 - Staining procedures.

Using a sterile loop take a loopful of homogenised sputum (see Section 4.5.1) and make a thin smear on a clean microscope slide for Gram staining.

Salivary specimens may be rejected before homogenisation or on the basis of a ratio of <2:1 WBCs: SECs determined by a Gram stain at low power magnification (x100).

If a specimen is rejected on the basis of microscopy inform the ward, clinician or GP immediately.

Retain specimens at 4°C for at least 48hr.

Note: Specimens from patients who are immunocompromised, neutropenic or intubated or for culture of *Mycobacterium* species should not be rejected on the basis of the quality of specimen.

Microscopy for *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

KOH - Calcofluor white preparation for fungi (TP 39 – Staining procedures).

BAL

Indirect immunofluorescent antibody test for *P. jirovecii* using a commercial kit.

Legionella

Fluorescent staining technique.

Homogenised specimens.

Using a sterile pipette place one drop of homogenised specimen (see Section 4.3) on to a clean PTFE microscope slide.

Spread the drop with a sterile loop to make a thin smear for fluorescent staining.

Follow kit manufacturers' instructions.

4.5 Culture and investigation

4.5.1 Standard

Sputum

Inoculate 1µL loopful of the final dilution prepared in 4.3 to each type of media plate (see Section 4.5.2).

For CF and patients who are immunocompromised also inoculate 1µL of the more concentrated sputasol/sputum dilution on the same plates. The dilutions may be plated on to half plates to allow easier comparison of growth.

For patients with cystic fibrosis who have no previous *B. cepacia* colonisation, inoculate 100µL of the liquefied sputum onto a *B. cepacia* plate and spread inoculum over the entire surface of the agar plate[99].

BAL

Using a sterile loop inoculate each agar plate with the deposit of the centrifuge sample (see Q 5 – Inoculation of culture media for bacteriology).

Semi-Quantitative method

Centrifuged BAL is re-suspended in the fluid and three serial dilutions are made (1/10, 1/1000 and 1/100,000. Of these dilutions 0.1mL of each is plated out[97].

	Volume plated to blood and chocolate	Final dilution
Vortexed BAL sample	0.1mL	1:10
Dilute 0.1mL in to 9.9mL saline	0.1mL	1:1000
Dilute 0.1mL in to 9.9mL saline	0.1mL	1:100,000

Quantitate each morphotype present and express as a colony forming unit.

Alternatively a calibrated loop is used. For BAL fluids samples, quantitative calibrated loops designed for the delivery of 0.010 and 0.001 mL are used. After incubation, the colonies are counted on the plates and the number of CFU per millilitre is determined by multiplying the number of colonies by the dilution factor. When using calibrated loops it is important to verify the calibration of the loop. Calibrations should be performed with BAL fluid as the test solution and borderline quantitative culture results should be interpreted with knowledge of the inaccuracy values of the loop[100].

Note: Do not delay between diluting the specimen and inoculating agar plates.

Diagnostic thresholds are 10^5-10^6cfu/mL for bronchoscopic aspirates, 10^3cfu/mL for protected brush specimens and 10^4cfu/mL for BAL[40]. The diagnostic threshold may not be met if the infection has just started or if infectious bronchiolitis is present. Specimens from patients who have received antibiotics may also give false-negative results.

4.5.2 Supplementary

Legionella

Sputum

Inoculate plates directly with 0.1mL of digested sputum (see section 4.3).

Bronchoalveolar lavages

Centrifuge at a minimum of 2000 x g for 15 mins. Use the deposit as the inoculum.

For other respiratory tract specimens select any milky or blood stained portion, if present, for use as the inoculum.

Heavily contaminated specimens should be heat-treated and diluted to decrease the numbers of yeasts, pseudomonads and *Proteus* species and then re-cultured.

Dilution

Dilute the original specimen 1:100 in distilled water and re-culture.

Note: when diagnosing legionellae the use of urinary antigen test can prove useful[66,101].

Note: heat treatment does not improve diagnostic yield and is therefore not included in the document[71].

Fungi

Non-CF patients ie immunocompromised and others:

After treating with mucolytic agent if required, spin entire sample. Examine part of residue with KOH and calcofluor white staining and culture the remainder.

CF Patients

After treating with a mucolytic agent plate culture one aliquot of 10uL and one aliquot of 100uL and spread well over the plate. Spin the remaining sample and examine part of the residue with KOH and calcofluor white staining and culture the remainder.

Other

Mycobacterium species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

Molecular detection methods

Numerous pathogens can be detected in respiratory samples by nucleic acid amplification or polymerase chain reaction (PCR) methods[102]. The advent of real-time PCR has allowed diagnoses to be made in a few hours. Many tests are available as commercial kits. PCR methods are always quicker than conventional methods and are usually more sensitive as well, potentially having a significant impact on treatment decisions.

4.5.3 Culture media, conditions and organisms for BAL samples

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchitis	BAL	Chocolate agar*	35-37	5-10%	40-48hr	Daily	*H. influenzae*

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Chest infection Chronic obstructive airways disease Community-acquired pneumonia Hospital-acquired pneumonia		+ Bacitracin disc or incorporated in the medium		CO_2			*M. catarrhalis* *S. aureus* *S. pneumoniae* Other organisms in pure growth may be significant
		Sabouraud agar (Screw-capped Universals should be used If dimorphic fungi suspected)	35-37 42-44	air	5d‡ 5d‡	≥40hr	Fungi
		CLED or MacConkey agar	35-37	air	40-48hr	Daily	Enterobacteriaceae Pseudomonads

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchiectasis Cystic fibrosis	BAL	Mannitol Salt/Chromogenic Agar	35-37	air	40-48hr	Daily	*S. aureus*
Cystic fibrosis[29]	BAL	*B. cepacia* selective agar Broth or solid medium as per B40	35-37	air	5d	Daily for five days	*B. cepacia complex* *M. abscessus***
Pneumonia or flu like symptoms	BAL	*Legionella* selective agar***	35-37	Moist Atoms	Up to 10d	at 3d, 7d and 10d	*Legionella* species

Other organisms for consideration –

Mycobacterium species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

* If chocolate agar with bacitracin incorporated into the agar is used then blood agar incubated in 5-10% CO_2 must be included for the isolation of *M. catarrhalis* and *S. pneumoniae*[63]. It may be difficult to differentiate between specific morphology of Streptococci on chocolate agar and in these instances a blood agar may be considered.

** Testing for *M. abscessus* should be carried out on request or at a patients Annual Review[21-24].

*** Buffered cefamandole, polymyxin, anisomycin, α-ketoglutarate medium (BMPAα) or Buffered charcoal yeast extract, anisomycin agar (BCYE) [64-66]. It should also be noted that incubation in 2-5% CO_2 can enhance growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*. This low level of CO_2 will not affect the growth of *L. pneumophila,* but CO_2 levels higher than 5% may inhibit growth [64]. The incubation of plates in 2-5% CO_2 is not compulsory; this is only mentioned for laboratories that may want to use it to enhance the growth of *Legionella* species. If laboratories choose to use *Legionella* selective agar plates as supplementary media, its inclusion should be subject to the results of local validation.

‡Fungal culture may need to be prolonged (up to 6 weeks) if dimorphic fungal pathogens are suspected; in such cases the screw-capped bijoux bottles should be read at 40hr and then left in the incubator/cabinet.

4.5.4 Culture media, conditions and organisms for sputum specimens

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchitis Chest infection Chronic obstructive airways disease Pneumonia	Sputum	Chocolate agar* + Bacitracin disc or incorporated in the medium	35-37	5-10% CO_2	40-48hr	Daily	*H. influenzae* *M. catarrhalis* *S. aureus* *S. pneumoniae* Other organisms in pure growth may be significant
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Bronchiecta sis Cystic fibrosis Immunoco mpromised/ ITU	Sputum	CLED agar or MacConkey agar	35-37	air	40-48hr	Daily	Enterobacteriaceae Pseudomonads
		Mannitol Salt / Chromogenic Agar	35-37	air	40-48hr	Daily	*S. aureus*
		Sabouraud agar	35-37	air	40-48hr†	≥40hr	Fungi
Cystic fibrosis[29]	Sputum	*B. cepacia* selective agar Broth or solid medium as per B40	35-37	air	5d	Daily	*B. cepacia* complex *M. abscessus***
Mycological investigatio ns	Sputum	Sabouraud agar (Screw-capped Universals should be used if dimorphic fungi suspected)	35-37	air	40-48hr†	≥40hr	Fungi
Legionella suspected	Sputum	*Legionella* selective agar***	35-37	Moist Atoms	Up to 10d	at 3d, 7d and 10d	*Legionella* species
Other organisms for consideration - *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).							
*If chocolate agar with bacitracin incorporated in the agar is used then blood agar incubated in 5-10% CO_2 must be included for the isolation of *M. catarrhalis* and *S. pneumoniae*[63]. It may be difficult to differentiate between specific morphology of Streptococci on chocolate agar and in these instances a blood agar may be considered. ** Testing for *M. abscessus* should be carried out on request or at a patients Annual Review[21-24].							

*** Refer to the information on Table 4.5.3.

†Fungal culture may need to be prolonged (up to 6 weeks for *P. brasiliensis*) if clinically indicated; in such cases the screw-capped bijoux bottles should be read at ≥40hr and then left in the incubator/cabinet.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Burkoholderia and related species	species level (see ID 17 – Identification of *Pseudomonas* species and other non-glucose fermenters)
S. maltophilia	species level
Enterobacteriaceae	From community samples to coliform level From inpatients to species level
Klebsiella pneumoniae	species level
Moulds	genus level
H. influenzae	species level
M. catarrhalis	species level
N. meningitidis	species level
Pasteurella	species level
Pseudomonads	"pseudomonads" level
P. aeruginosa	mucoid or non-mucoid species level
S. aureus	species level
S. pneumoniae	species level
Yeasts	"yeasts" level
Legionella	species level
Mycobacterium	see B 40 - Investigation of specimens for *Mycobacterium* species
Parasites	see B 31 - Investigation of specimens other than blood for parasites

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for outbreak Investigations

N/A

4.9 Referral to reference laboratories

Legionella species obtained from clinical material must be referred for identification and serogrouping.

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm

5 Reporting procedure

5.1 Microscopy

If the patient is immuno-competent, report poor quality or salivary specimens as:

"Poor quality specimen/salivary specimen received. Please repeat if clinically indicated".

Gram stain (if performed).

Report on epithelial cells, WBCs and organisms detected.

Report on fungal hyphae detected.

Legionella pneumophila detected by immunofluorescence or

Legionella pneumophila not detected by immunofluorescence.

P. jirovecii immunofluorescence

P. jirovecii cysts detected by immunofluorescence or

P. jirovecii cysts NOT detected by immunofluorescence.

Microscopy for *Legionella, Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

Report clinically significant organisms isolated and their amount if BAL and semi-quantitative method employed or

Report other growth, eg: Mixed upper respiratory tract flora or

Report absence of growth or

Report absence of growth of specifically targeted organism at a 10^{-6} dilution of the specimen (for CF and bronchiectasis patients)

Report results of supplementary investigations.

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Supplementary investigations, *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[103,104], or equivalent in the devolved administrations[56-59]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

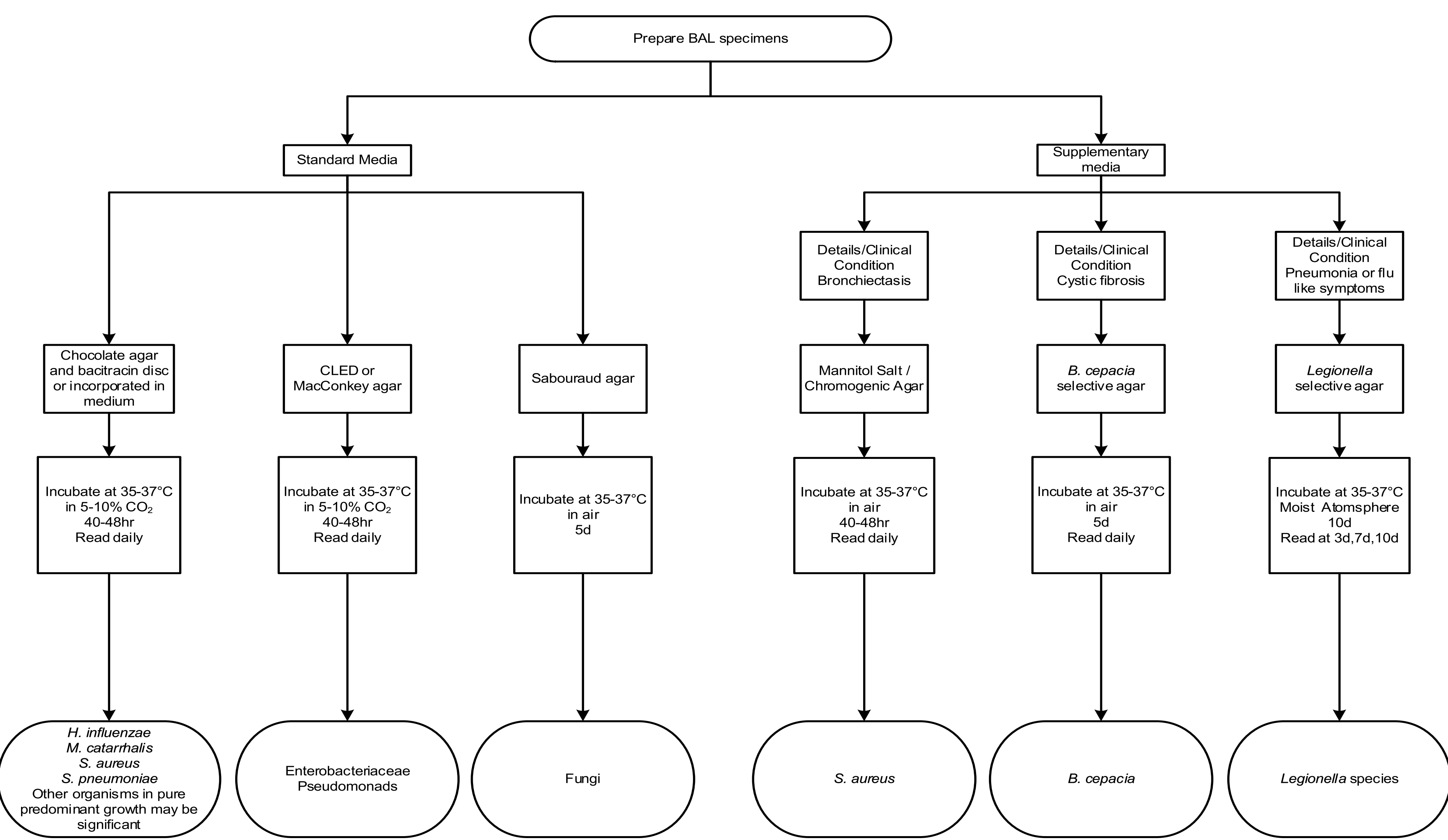
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

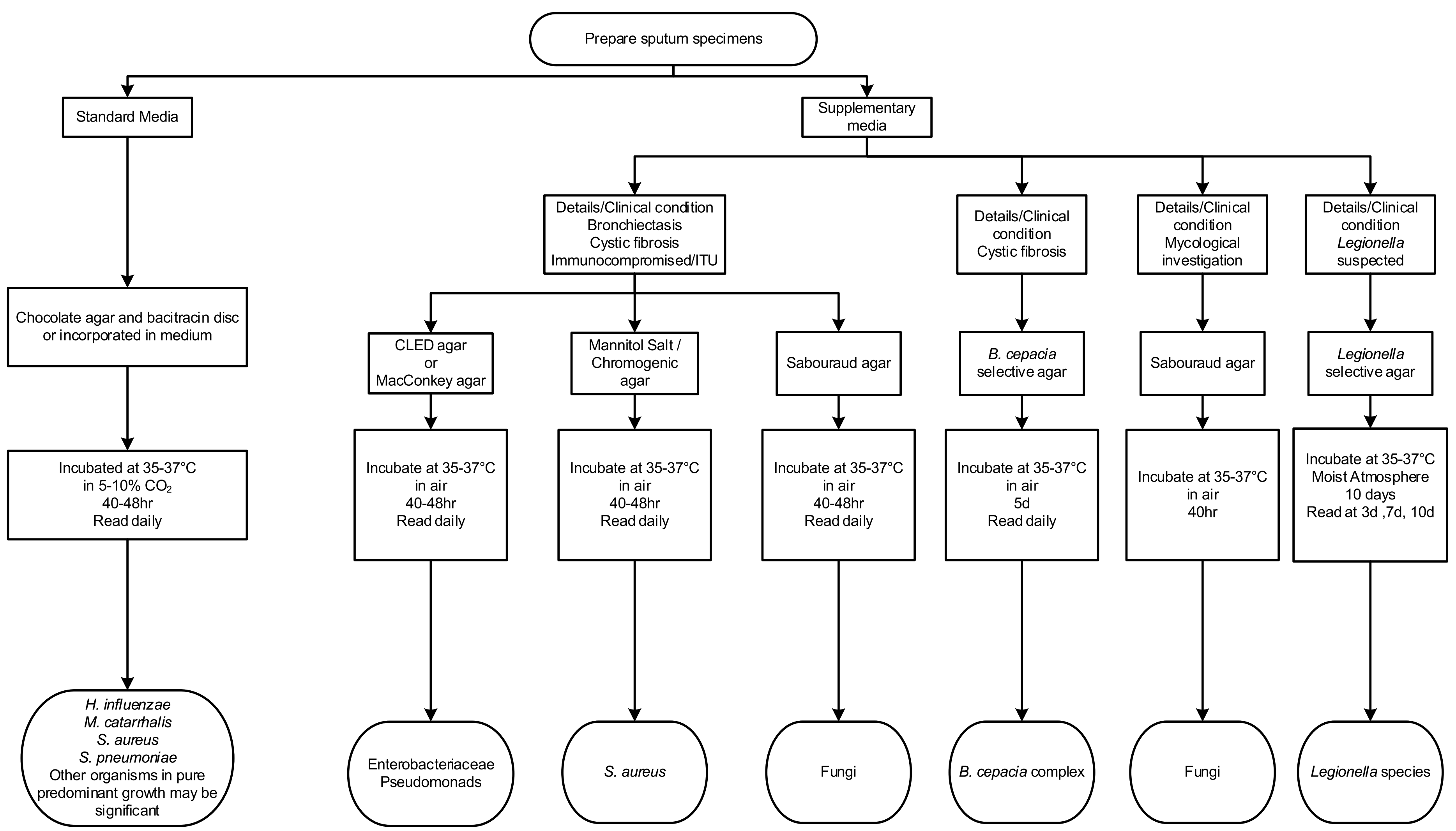
https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[56,57], Wales[58] and Northern Ireland[59].

Appendix 1: BAL specimens for culture



Appendix 2: Sputum specimens for culture



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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working group for microbiology standards in clinical bacteriology

B 57 Investigation of bronchoalveolar lavage, sputum and associated specimens

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation 28.01.13 – 22.04.13

Version of document consulted on – B 57dd+

Proposal for changes

Comment number	1		
Date received	10/04/2013	**Lab name**	Imperial College Healthcare NHS Trust
Section	2.5.1		
Comment			
Routine dilution of sputum samples was recommended in this guideline. Only one reference (no. 57) which was published by Dixon and Miller in 1965 was used to support this method. Are there any evidence-based and more recent publications in the medical literature to back this method up?			
Recommended action	**ACCEPT** This section of the document has been re written and updated.		

Comment number	2		
Date received	22/04/2013	**Lab name**	Mycology Reference Laboratory, Bristol
Section	Various		
Comment			
Overall Suggest that we need a section specifically related to mycological diagnosis where we make different recommendations for fungal culture from different patient groups. When there is little fungus present in the BAL or sputum (immunocompromised setting) you can maximise isolation and microscopic detection by processing the entire sample. Whereas with the CF patients if you process the entire sample you grow the majority organism which is usually *Aspergillus fumigatus* but you often miss accompanying *Scedosporium* and *Exophiala* species. Non-CF patients ie immunocompromised and others: After treating with mucolytic agent if required, spin entire sample. Examine part of residue with KOH and calcofluor staining and culture the remainder. CF patients: After treating with a mucolytic agent plate 1uL and spread well over plate. Spin the remainder and examine part of the residue with KOH and calcofluor staining and culture the remainder. a. There is no mention of the fact that respiratory samples from patients with travel history to areas where dimorphic fungi are endemic, however long ago, may grow such fungi. Any mould grown from such a patient should be processed at CL3 as			

soon as it is detected, and until a dimorphic fungus is excluded. I think this is a significant missed safety opportunity! Page 7: scope should include fungal respiratory infection.

b. Page 11, line 3: spelling of coccidioidomycosis.

c. Page 11, line 4: spelling of Coccidioides plus change *C. pedrosii* to *C . posadasii.*

d. Page 11: Add *Penicillium marneffei* (South east Asia, southern China) and *Blastomyces dermatitidis* (North America, Central and South America and Africa).

e. Page 11: *Crytococcus neoformans* and *C. gatti*i are unusual causes of pneumonia in immunocompetent individuals and are mainly encountered in HIV-infected individuals. These need to be distinguished from commensal Candida species.

f. Page 11 Paracoccidioidomycosis caused by *Paracoccidioides brasiliensis* (Central and South America) usually causes asymptomatic primary pulmonary infection that may reactivate if immune function declines. This applies to all the fungi mentioned above not just Paracoccidioidomycosis.

g. Page 13 Under 'Technical information/limitations': - 'Mucorales' should be 'mucoraceous moulds'.

h. Page 14 2.1 containment level 3 - not clear if this is for all respiratory samples.

i. Page 14 2.2 Test selection/Additional comments for BAL: - Patients considered to be at risk of pulmonary aspergillosis, or in whom fungal infection is suspected, should have a portion of BAL fluid tested for Aspergillus galactomannan (or perhaps this goes in 2.5 before Molecular methods).

j. Page 14 2.2 Induced sputum may be sent for investigation for *P. jirovecii* --- (B 31 - Investigation of specimens other than blood for parasites). *P. jirovecii* is a fungus.

k. Page 15 2.4.2 Gram staining may identify yeasts or (not of) hyphae

l. Page 16 2.4.2 Salivary specimens may be rejected before homogenisation or on the basis of a ratio of <2:1 WBCs:SECs determined by a Gram stain at low power magnification (x100). - Need a reference.

m. Page 16 2.4.2 KOH preparation or Calcofluor for fungi.

BAL

Indirect immunofluorescent antibody test for *P. jirovecii.*

Where are the sections for these?

n. Page 16 2.5.1 Dilute 10µL of homogenised sputum in 5mL of sterile distilled water.

This is small sample when at least 1.0ml was recommended earlier - will miss fungi.

Page 17 Supplementary

o. Fungi, Mycobacterium species (B 40 - Investigation of specimens for Mycobacterium species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

The fungi section seems to have been missed.

Page 18 Table 2.5.2

p. Why are Sab plates read at 40 hours?

Where it says 'Mycological investigations' we suggest that SAB is cultured at both 35-37C and 42-44C for 5 days. q. Footer suggest: ‘ Keep cultures up for longer (up to six weeks) if dimorphic fungal pathogens are suspected’ ...... delete specific mention of *P. brasiliensis.* Page 20 2.6 Minimum level of ID r. 'Fungi' and 'Yeasts' are listed separately (this is universal in SOPs I think). This should either say 'Yeasts' and 'Moulds' (preferred) or just 'Fungi'. s. Page 21 Notification to the PHE.

Recommended action	a. **ACCEPT** This part of the document has been strengthened. b. **ACCEPT** Correction made. c. **ACCEPT** Correction made. d. **ACCEPT** These fungi have been added to the document. e. **ACCEPT** Candida has been moved out of the more unusual fungal causes part of the document. f. **ACCEPT** Sentence moved and made more general. g. **ACCEPT** Wording changed. h. **NONE** Document refers to level 3. i. **ACCEPT** Section inserted in to 2.2. j. **ACCEPT** Cross reference removed. k. **ACCEPT** Correction made. l. **NONE** This is accepted as good practice and is not documented m. **ACCEPT** These sections of the document have been strengthened.

	n. **ACCEPT** This will be covered in the strengthening of the fungal section. o. **ACCEPT** This will be covered in the strengthening of the fungal section. p. **ACCEPT** Document amended. q. **ACCEPT** Document amended. r. **ACCEPT** All UK SMIs will be changed to Yeasts and Moulds as part of PHE rebranding exercise. s. **ACCEPT** All UK SMIs will be changed as part of PHE rebranding exercise.

Comment number	3		
Date received	18/11/2013	**Lab name**	University Hospital Limerick,
Section			
Comment			
We follow the PHE guidance for the processing of process our sputa and cystic fibrosis specimens- HPA SOP 57 Investigation of Bronchoalveolar Lavage Sputum and Associated Specimens. However, we have learned that some centres use an additional cetrimide agar and incubate for up to 5D to increase detection of Pseudomonas. Can you advise if there are any plans to revise the PHE guidelines as our issue is that we have recently missed detection of Pseudomonas as a consequence of partaking in an EU study. I don't believe NEQAS utilises specific resp. samples from the CF population?			
Recommended action	**NONE** The document recommends the minimum requirement. Additional plates can be added depending on local requirements.		

2nd Consultation 02.06.14 – 26.08.14

Version of document consulted on – B 57dr+

Proposal for changes

Comment number	1		
Date received	02/06/2014	**Lab name**	PHE/RCPath
Section	a. Pneumonia section Community acquired subsection p8 b-e. Cystic Fibrosis/ Fungal Infection c. 5.1 Microscopy/ f-j. General Comments		

Comment

a. *Chlamydia pneumoniae*, *Chlamydia psittaci* should be *Chlamydophila pneumoniae*, *Chlamydophila psittaci*.

In the line 'Respiratory viruses, such as Respiratory syncytial virus (RSV), influenza and adenoviruses may occasionally cause primary viral pneumonia' suggest lower case 'r' for respiratory syncytial virus, and remove 'occasionally'. Respiratory viruses are thought to cause at least 10% of CAP in adults and more in children, so you might replace 'occasionally' with 'commonly'; in the study from Canada cited as evidence below viruses alone were found in 15%, bacteria alone 20%.

CF section:

b. *H. influenza* should be *H. influenzae*.

c. The importance of viruses in exacerbations of CF might be mentioned.

d. *Mycobacterium abscesses* infection might be included in this section (or the next)

Fungal Infection section:

e. BAL is a good specimen for *P. jirovecii* PCR as is sputum (induced or expectorated).

f. Microscopy: *P. jirovecii* 'oocysts' - should be 'cysts'.

Additional Comments:

g. Some suggestion of considering viruses should be added to the algorithms.

h. There is no mention of *B. pertussis*.

i. Should there be a paragraph about other less common pathogens such as Q fever and dangerous pneumonia pathogens such as *B. anthracis*?

j. Wouldn't 'calcofluor white' be preferable to 'calcofluor' throughout document?

k. The method of aspergillus culture should be specified, suggest using the Manchester Mycology Reference Centre methodology to increase yield.

Evidence

Johnstone J et al Viral infection in adults hospitalized with community acquired pneumonia. Chest 2008; 134: 1141-8

Fraczek MG et al. Volume dependency for culture of fungi from respiratory secretions and increased sensitivity of Aspergillus quantitative PCR Mycoses 2014;57:69-

78.Fraczek MG(1), Kirwan MB, Moore CB, Morris J, Denning DW, Richardson MD	
Financial barriers	
No.	
Recommended action	a. **ACCEPT** Amended. b. **ACCEPT** Amended. c. **ACCEPT** Amended. d. **ACCEPT** Placed in the document where applicable. e. **ACCEPT** The document has been amended and references added. f. **ACCEPT** Amended. g. **ACCEPT** References have been added. h. **ACCEPT** A cross reference to B 6 – Culture of specimens for *Bordetella pertussis* and *Bordetella parapertussis*. i. **ACCEPT** The document has been amended to include this information. j. **ACCEPT** This has been changed throughout the document and brought in line with TP 39. k. **PARTIAL ACCEPT** The reference has been inserted in to the document.

Comment number	2		
Date received	03/06/2014	**Lab name**	Wexham Park Hospital
Section	Appendix (both)		
Comment			
Standard media states chocolate plus bacitracin disc or incorporated into medium.			

Should add blood agar plate as standard if it is incorporated in order to isolate *S. pneumoniae*.	
Evidence	
Referred to in section 4.5.3.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	**ACCEPT** This media has been added as an option.

Comment number	3		
Date received	26/08/2014	**Lab name**	UKCMN
Section	Introduction - fungal infection		
Comment			
a. Paragraph 2 Line 1 - Aspergillosis should be aspergillosis. b. Line 2 - contribute should be contributes. c. Paragraph 5 Line 3 - need a space between in and immunocompetent. d. Line 4 - individuals but reported should be are reported. e. Line 8 - need to add Africa, Australia and eastern Asia after Central America. f. Line 9 - Coccidioide simmitis should be Coccidioides immitis. g. Line 10 - should be eastern USA, Central and South America and Africa. h. Line 14 - Talaromyces (previously Penicillium) marneffei. i. Line 14 - Blastomyces is already mentioned above in the paragraph. j. Paragraph 6 Line 1 - should be immunocompromised host. k. Line 4 - Circulating antigen in the serum or BAL.			
Recommended action	**ACCEPT** These edits (a-k) have been accepted and made.		

Comment number	4		
Date received	26/08/2014	**Lab name**	Cambridge PHE
Section	a. 3		

	b. 4
Comment	

a. Delays in processing are a particular concern for respiratory samples where the CFU of potential pathogens such as *Haemophilus influenzae* and *Strep pneumoniae* may decrease if left at fridge temperature and organisms such as the pseudomonads may multiple at room or even fridge temperature. A BAL is not undertaken lightly and should be transported to the laboratory promptly and processed optimally (I appreciate it is difficult to set standards for this and this needs local agreement). This is becoming more of an issue with the centralisation of microbiology services and the resulting delays in processing. Suggest clearer wording such as: 'BAL and sputum should be processed promptly to give the best opportunity to culture pathogenic organisms and reduce the risk of overgrowth with contaminants. If processing has to be delayed, refrigeration (of up to 24 hours) is preferable to storage at ambient temperature. If specimens are not processed on the same day that they are collected, this should be noted on the report and interpretation of results should be made with care.'

b. 4.3 Why 15 minutes DTT at 35-37C, many labs use room temperature and leave until homogenised is that no longer acceptable?

c. 4.4.2 PCP what about a PCR method (not my area of expertise so unable to give evidence but I am sure that others will have commentated).

d. 4.5.3 This is not the same as the summary in Appendix 1 and 2. For example: 1. Different incubation temperatures quoted for culture of Bcc.2. MSA for *Staph aureus* for CF in Appendix but not in section.

e. 4.5.3. 3. Different incubation times for fungi.

f. If no blood agar plate is recommended, are you confident that staff will recognise beta haemolytic streptococci? - group A strep are an important cause of serious post flu pneumonia. Will staff be able to recognise *S. pneumoniae* on chocolate agar - when they may appear indistinguishable from viridians-type streptococci?

g. 4.6.1 Identification only refers to *Burkholderia cepacia* complex. Should also include reference to *B. gladioli* (which is not part of the *Burkholderia cepacia* complex), also to *B. pseudomallei*. Suggest change *Burkholderia cepacia* complex in left hand column to *Burkholderia* spp., with recommendation to identify to species level as already stated 'pseudomonads' is not a suitable level of identification for CF or bronchiectasis patients. Unusual morphotypes of *P. aeruginosa* may be missed. Organisms such as *Ralstonia*, *Achromobacter* and *Pandoraea* are emerging pathogens in chronic structural lung disease. Suggest a minimum of identification to genus level.

Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **ACCEPT** This section has been updated.

	b. **ACCEPT** Now say follow manufacturer's instructions. c. **ACCEPT** This has now been mentioned. d. **ACCEPT** These have now been brought in line. e. **NONE** The second temperature is for universal tubes not plates. f. **ACCEPT** A note on this situation and the suggestion of an addition of a blood plate have been added. g. **ACCEPT** This information has now been included in the introduction and the minimum level of identification section modified.

Comments received outside of consultations

Comment number	1		
Date received	31/12/2012	**Lab name**	HPA Public Health Laboratory Manchester
Section	Whole document		
Comment			
Incidentally where *P. jirovecii* is discussed in the SMIs, eg in B57, it is misspelt as *P. jiroveci* - this is a common mistake as it was the first nomenclature used but please correct it.			
Evidence			
If you want the reason see Stringer JR, Beard CB, Miller RF. Spelling *Pneumocystis jirovecii*. Emerg Infect Dis. 2009 March; 15(3): 506.			
Recommended action	**ACCEPT** Change made.		

Comment number	2		
Date received	07/01/2013	**Lab name**	MSTAG
Section	a. Molecular Detection methods b. Semi-quantative culture		

	c. Vortexing d. 2.5.2 e. 2.5.3 f. Whole document
Comment	
a. “extraction systems are specific”- not true. b. Confusing-needs simplifying. Needs clarification which is for Sputa/BAL. c. Although it affects the “air-curtain”, it is just as risky to vortex outside-ie what happens if tube breaks. Also the term “curtain” is this a reference to a Type 2 cabinet as type 1s do not have an air curtain. d. i. Blood agar not in table any more, what happened to this as a basic media for minimum standards, either incubated CO_2 or anaerobically. ii. Supplementary media-add chromogenic staph media as well as mannitol salt agar. iii. Although Legionella is a separate method add in Legionella media here. iv. *B. cepacia* agar confusing, should read “35° C for 2 days then 30° C for 3 days not 5 days. e. This table is so similar to 2.5.2, is it required? f. General comment not necessarily relevant to this method but it was noted that pathogenic fungi can grow in liquid TB media.	
Recommended action	a. **ACCEPT** Sentence removed. b. **ACCEPT** Section has been streamlined. c. **ACCEPT** Sentences removed. d. i. **NONE** Blood agar has never been in this document as we have a chocolate agar plate. ii. **ACCEPT** This media option has been added to the document. iii. **ACCEPT** The media has been added. iv. **ACCEPT**

	The times in the table have been clarified. e. **NONE** There are subtle differences which would be lost if the tables were merged. f. **NONE**

Respondents indicating they were happy with the contents of the document

Overall number of comments: 7			
Date received	29/01/2013	**Lab name**	SRM Institute For Medical Sciences, Chennia, India
Date received	31/01/2013	**Lab name**	RIE
Date received	07/02/2013	**Lab name**	Ex Laboratorio Microbiologica Careggi Firenze
Date received	13/02/2013	**Lab name**	Golden Jubilee National Hospital
Date received	15/02/2013	**Lab name**	Microbiology, Newcastle Hospitals NHS Trust
Date received	03/06/2014	**Lab name**	Microbiology
Date received	10/06/2014	**Lab name**	Princess of Wales' hospital

UK Standards for Microbiology Investigations

Detection of Carriage of Group B Streptococci (*Streptococcus agalactiae*)

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 58 | Issue no: 3.1 | Issue date: 26.06.18 | Page: 1 of 24

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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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Contents

ACKNOWLEDGMENTS **2**

AMENDMENT TABLE **4**

UK SMI: SCOPE AND PURPOSE **7**

SCOPE OF DOCUMENT **10**

INTRODUCTION **10**

TECHNICAL INFORMATION/LIMITATIONS **13**

1 SAFETY CONSIDERATIONS **14**

2 SPECIMEN COLLECTION **14**

3 SPECIMEN TRANSPORT AND STORAGE AND RETENTION **15**

4 SPECIMEN PROCESSING/PROCEDURE **15**

5 REPORTING PROCEDURE **18**

6 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS **18**

APPENDIX: DETECTION OF CARRIAGE OF GROUP B STREPTOCOCCI (S*TREPTOCOCCUS AGALACTIAE*) **20**

REFERENCES **21**

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	7/26.06.18
Issue no. discarded.	3
Insert Issue no.	3.1
Section(s) involved	**Amendment**
Whole document.	Title amendment to add "*Streptococcus agalactae*". Content, references and hyperlinks updated to reflect current policy of the UK National Screening Committee and guidance from the National Institute for Health and Care Excellence and the Royal College of Obstetricians & Gynaecologists which recommends the circumstances in which the screening may be helpful in determining the risk of developing early-onset neonatal GBS (EOGBS) or in examining cases of late-onset neonatal GBS. Specimen updated to be more specific to maternal samples.
1.1 Specimen collection, transport and storage.	Amies or Stuart medium for transport added.
4.5.1 Culture media, conditions and organisms.	Gentamicin and nalidixic acid combination added as a LIM Broth option.
4.7 Antimicrobial susceptibility testing.	Antimicrobial susceptibility testing and reporting table added.
4.9 Referral to reference laboratories.	Updated sentence to: "Refer all GBS which are associated with an infection control or cluster investigation in addition to all invasive isolates."
Appendix.	Updated.

Amendment No/Date.	6/19.06.15
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Emphasis on availability of this GBS detection method for laboratories to undertake when required and does not cut across the UK National Screening Committee recommendation that antenatal screening for GBS colonisation is not recommended. Any mention of screening pregnant women for colonisation of GBS has been removed to minimise ambiguity.
Title.	The title of the document has been changed from ‘Processing swabs for Group B streptococcal carriage’ to ‘Detection of carriage of Group B streptococci’.
Scope.	Amended to make the scope and purpose of the document clear.
Introduction.	Re-structured to present the information clearly.
Colonisation.	Colonisation of GBS amended from up to 30% to up to 28% and referenced.
Infection.	Updated. Reference to a 1998 Working Group removed.
Method of investigation.	Updated. The use of selective agar for subculture from enrichment broth added. Collection of recto-vaginal swabs between 35 and 37 weeks gestation and USA guidelines has been removed.
Treatment.	The section is outside the scope of the document and has been removed.
Rapid methods.	Section added.
Specimen collection.	Updated for clarity. Type of manufactured swabs covered. Collection of specimens by qualified caregiver or patient added.

Culture and investigation.	Updated to provide information on culture examination.
4.5.1 Culture media, conditions and organisms.	Updated to include options for selective and chromogenic agar for subculture of enrichment broth.
Appendix.	Flowchart added.
References.	Updated.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratorieshttp://www.hpa-standardmethods.org.uk/. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

Public Health England. (2018). Detection of Carriage of Group B Streptococci (Streptococcus agalactiae). UK Standards for Microbiology Investigations. B 58 Issue

3.1. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Maternal low vaginal and rectal swabs

Scope

The method describes the examination of specimens to detect carriage of group B streptococci (GBS). This method may also be of value to support research projects investigating GBS carriage.

The recommendations within the UK SMI recognise the current policy of the UK National Screening Committee (2017) and guidance from the National Institute for Health and Care Excellence and the Royal College of Obstetricians & Gynaecologists (2017) which states that routine universal antenatal screening using bacteriological culture or near patient testing techniques should not be introduced in UK practice[1-3]. However, current guidance recommends that in certain settings, screening may be helpful in determining the risk of developing early-onset neonatal GBS (EOGBS) or in examining cases of late-onset neonatal GBS[4]. For example, in women in whom GBS was detected in a previous pregnancy, administration of intrapartum antibiotic prophylaxis (IAP) provides a clear clinical benefit[5]. This UK SMI provides details on the test method in the event of undertaking the screening of an individual mother.

On rare occasions, clusters of invasive GBS infection occur. In such circumstances, non-invasive sampling of cases or screening of individuals at risk, contacts, or the environment may assist with outbreak management[4].

This UK SMI does not include procedures for isolation and detection of GBS from invasive specimens such as blood and CSF; refer to the UK SMIs B 37-Investigation of blood cultures (for organisms other than *Mycobacterium* species) and B 27-Investigation of cerebrospinal fluid. However, during investigation of a maternal or neonatal invasive GBS infection or cluster, additional carriage and screening swabs may be taken from infants, mothers and the environment as part of the investigation.

The testing of environmental samples is outside of the scope of this UK SMI.

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

Lancefield group B streptococci

Lancefield group B streptococci (GBS), or *Streptococcus agalactiae*, are facultatively anaerobic, oxidase-negative, catalase-negative, Gram-positive cocci occurring in chains. GBS are serologically classified on the basis of cell wall polysaccharide antigens and exhibit β-haemolysis on blood agar, although a very small proportion of strains are non-haemolytic. These characteristics can be used as an early step in identifying clinical isolates. After 18-24 hours incubation at 35-37°C colonies tend to be slightly larger than other streptococci (approximately 1mm) and have a less distinct zone of β-haemolysis (see ID 4 – Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms).

Colonisation

GBS normally colonises the vagina in many women and the intestines of men and women. Up to 28% of women in the UK carry GBS in the vagina or rectum without any associated symptoms[6-8]. The gastrointestinal tract is the human reservoir for GBS and the likely source of vaginal colonisation[9].

Infection

Although GBS colonisation is not normally associated with disease in non-pregnant women, GBS can cause infection including bacteraemia in pregnant women[9]. GBS may cause potentially devastating early onset disease, primarily in newborns and late onset disease in newborns as well as infections in pregnant women, children and adults. In pregnancy this organism can infect the amniotic fluid (see B 26 – Investigation of fluids from normally sterile sites) which can lead to neonatal sepsis, pneumonia or meningitis[10].

In pregnant women, GBS infection is known to cause urinary tract infection, amnionitis, endometritis and wound infection. In men and non-pregnant women, skin or soft tissue infection, bacteraemia, genitourinary infection, balanitis (in men) and pneumonia are the most common manifestations of disease[11,12].

Neonatal infection refers to infection occurring during the first four weeks of life. Infection may be superficial and localised (eg conjunctivitis, pustules, skin infection), deep and localised (pneumonia, septic arthritis, meningitis) or systemic (septicaemia). Presentation differs according to age at onset: early onset disease is more likely than late onset to present with generalised sepsis[13].

Since The British Paediatric Surveillance Unit (BPSU) study of 2000–2001 there has been a significant increase in the incidence of invasive GBS disease in all five British Isles countries. Results from a repeat of this study in 2014, showed the incidence for early-onset GBS disease was 0.54 cases per 1000 live births and a mortality rate of 4.7% compared to 0.48 cases per 1000 live births and a mortality rate of 9.7% in 2000[13,14]. Increases in erythromycin and clindamycin resistance have also been noted over this period, leading to a change in second line agent used for intrapartum prophylaxis[15].

The incidence of infection increases with low birth weight or prematurity and may be divided into:

- Early onset (0-6 days) - this occurs in the first six days (usually within 48 hours) of life and is caused by infection ascending from the maternal genital tract or, very rarely, via the placenta. Only a small percentage of infants colonised with this organism develop early onset disease. Early infections tend to be associated with pneumonia and septicaemia and may be confused with respiratory distress syndrome
- Late onset (7-90 days) - this occurs after the first six days (7-90 days) and is associated with acquisition of the organism through vertical or nosocomial transmission or from the external (eg hospital) environment. GBS initially colonise the superficial sites and upper respiratory tract and progress to cause widespread sepsis. Late infection is more likely to be associated with meningitis

In the UK, universal antenatal screening for GBS colonisation is currently not recommended[1-3,5,16]. However, the Royal College of Obstetricians & Gynaecologists recommends screening in women in whom GBS was detected in a previous

pregnancy at 35-37 weeks of gestation or 3-5 weeks prior to the anticipated delivery[1]. This screening would determine the carriage status close to delivery and provides information that helps to assess the risk of EOGBS. Based on the results of this screening, IAP can be offered and in the case of mothers with clinical risk factors they can chose to decline IAP if they test negative.

Method of investigation

The isolation rate of GBS from clinical specimens depends on several factors. Studies have shown that detection for GBS colonisation can be improved by attention to the timing of cultures, the sites swabbed and the microbiological method used for culture of microorganisms. The Centers for Disease Control and Prevention suggest that optimum yield will be achieved by selective enrichment procedures applied to swabs obtained from the vagina and the anorectum which increases the likelihood of GBS isolation compared with vaginal or cervical culture alone[17]. Recto-vaginal swabs are likely to isolate a diverse array of normal microflora and use of selective enrichment broth is recommended to avoid overgrowth of other microorganisms[17].

The use of a selective enrichment broth that inhibits the growth of competing organisms such as Gram negative enteric bacilli and other normal microflora significantly increases the yield of GBS culture and is recommended since it has been found to be the most sensitive method to detect female colonisation[17,18]. The most widely used selective enrichment broth is Todd-Hewitt broth with nalidixic acid and colistin (eg Lim broth) or nalidixic acid and gentamicin, with further subculture on blood agar plate.

Subculture from the selective enrichment broth to a selective and chromogenic agar have deomonstrated equivalent or superior GBS recovery compared to subculture to blood agar[18-20].

Chromogenic media are not fully specific for GBS identification and presumptive colonies of GBS should be confirmed by a specific antigenic detection test or Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[21-24].

Rapid methods

A variety of rapid identification methods are available to detect GBS presence in pregnant women directly from vagino-rectal swabs, of which some are FDA approved. Assay sensitivity of some tests is reported to be higher than culture alone[25]. However, discordant results are noted and in some cases results are variable on repeat testing. Use of broth enrichment followed by subculture on most chromogenic media and PCR assays have comparable sensitivities and allow more rapid reporting of screening for GBS than conventional culture methods[20].

For presumptive isolates of GBS a variety of rapid identification methods are available with high sensitivity and specificity including PCR and Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)[21,22]. Refer to ID 4 - Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms for the identification of GBS.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[26,27]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

1 Safety considerations[26-42]

1.1 Specimen collection, transport and storage[26-31]

Use aseptic technique.

Collect swabs into appropriate transport medium eg Amies or Stuart, and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[26-42]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[34].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Maternal low vaginal and anorectal swabs. Maternal high vaginal swabs should not be collected as these have a lower sensitivity.

2.2 Optimal time and method of collection[43]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[43].

It is essential to specify “Detection of GBS carriage” in the specimen request.

Unless otherwise stated, swabs for GBS culture should then be placed in appropriate transport medium[44-48].

Rayon or Dacron, Fibre or Flocked swabs, with non-nutritive transport media (eg Amies or Stuart’s), preserve the viability of the organism by providing moisture, and buffering to maintain the pH.

Specimen(s) for culture may be collected either by the physician or other qualified caregiver (or may be self-collected by the patient, with appropriate instruction). This involves swabbing the distal vagina (vaginal introitus), followed by the rectum.

A single swab for both sites of collection is rational, but two different swabs can be used. Because lower vaginal as opposed to cervical cultures are recommended, cultures should not be collected by speculum examination.

2.3 Adequate quantity and appropriate number of specimens[43]

One combined maternal vaginal/rectal swab or two separate swabs processed as one.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen transport and storage and retention[26,27]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[43].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[43].

GBS isolates can remain viable in transport media for several days at room temperature. However, the recovery of isolates declines over 1-4 days, especially at elevated temperatures, which can lead to false-negative results. Specimens should be refrigerated before processing[17].

4 Specimen processing/procedure[26,27]

4.1 Test selection

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

N/A

4.5 Culture and investigation

Selective enrichment culture

Remove the cap aseptically from the container and place the swab(s) in the LIM broth, break off (or cut) the swab stick(s) and replace the cap. Caps should be kept loose during incubation.

Culture

After an overnight incubation at 35-37°C, 5% CO_2, subculture with a sterile loop and inoculate appropriate media (see table 4.5.1).

Optimum detection of GBS may require the use of more than one culture medium.

For the isolation of individual colonies, spread inoculum with a sterile loop onto blood agar, selective or chromogenic agar.

Incubate the plate(s) at 35 to 37°C in the appropriate atmosphere for 24-48hr.

Culture examination

After an overnight incubation, observe plates for suggestive GBS colonies and identify them. If negative after overnight incubation, re-incubate an additional 24 hours before reporting a negative result.

On blood agar, suggestive colonies of GBS are grey, translucent, with a surrounding zone of beta-hemolysis (or no hemolysis: very rare).

Refer to manufacturer's instructions for GBS detection on selective and chromogenic agar.

Serotyping of isolates is available by latex agglutination or on referral to the reference laboratory if from invasive or associated to invasive cases.

4.5.1 Culture media, conditions and organisms

Clinical details/ Conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Carriage of Group B streptococci	Maternal low vaginal and anorectal swabs	LIM Broth (5mL)†: Todd-Hewitt broth supplemented with 10µg/mL colistin - or 8µg/mL gentamicin and 15µg/mL nalidixic acid	35-37	5% CO_2	18-24hr	N/A	
		Then subculture to: Blood agar or	35-37	5% CO2	24-48hr	18-24hr and 48hr	Group B streptococci
		Selective agar or	35-37	Ambient	24-48hr	18-24hr	
		Chromogenic agar	35-37	Ambient	24-48hr	18-24hr	

†The bottle should contain a volume of broth sufficient to cover the swabs

4.6 Identification

Refer to ID 4 - Identification of Streptococcus species, Enterococcus species and morphologically similar organisms for the identification of GBS.

4.6.1 Minimum level of identification in the laboratory

Streptococcus agalactiae	species level

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Refer to EUCAST guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

This UK SMI recommends selective and restrictive reporting of susceptibilities to antimicrobials. Any deviation must be subject to consultation that should include local antimicrobial stewardship groups.

4.7.1 Antimicrobial Susceptibility Testing and Reporting Table

It is recommended that the antimicrobials in bold in the table below are reported. Those antimicrobials not in bold may be reported based on local decisions.

Bacteria	Examples of agents to be included within primary test panel (recommended agents to be reported are in bold depending on clinical presentation)	Examples of agents to be considered for supplementary testing (recommended agents to be reported are in bold depending on clinical presentation)	Notes
GBS	**Penicillin** **Clindamycin** **Cefotaxime** **Vancomycin**	Clarithromycin **Teicoplanin**	After consultation with users, laboratories may report only Penicillin routinely and report the other agents routinely only if shown to be resistant.

4.8 Referral for outbreak investigations

See 4.9.

4.9 Referral to reference laboratories

Refer all GBS which are associated with an infection control or cluster investigation in addition to all invasive isolates.

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms (England and Wales).

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

N/A

5.2 Culture

Report:

Negatives

"Group B streptococci not isolated"

Positives

"Group B streptococci isolated"

5.2.1 Culture reporting time

Clinically urgent results: to be telephoned or sent electronically.

Written report: 16 – 72hr stating, if appropriate, that a further report will be issued.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[49,50] or equivalent in the devolved administrations[51-54]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days. GBS is a notifiable disease in Northern Ireland but not in England, Wales and Scotland.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many

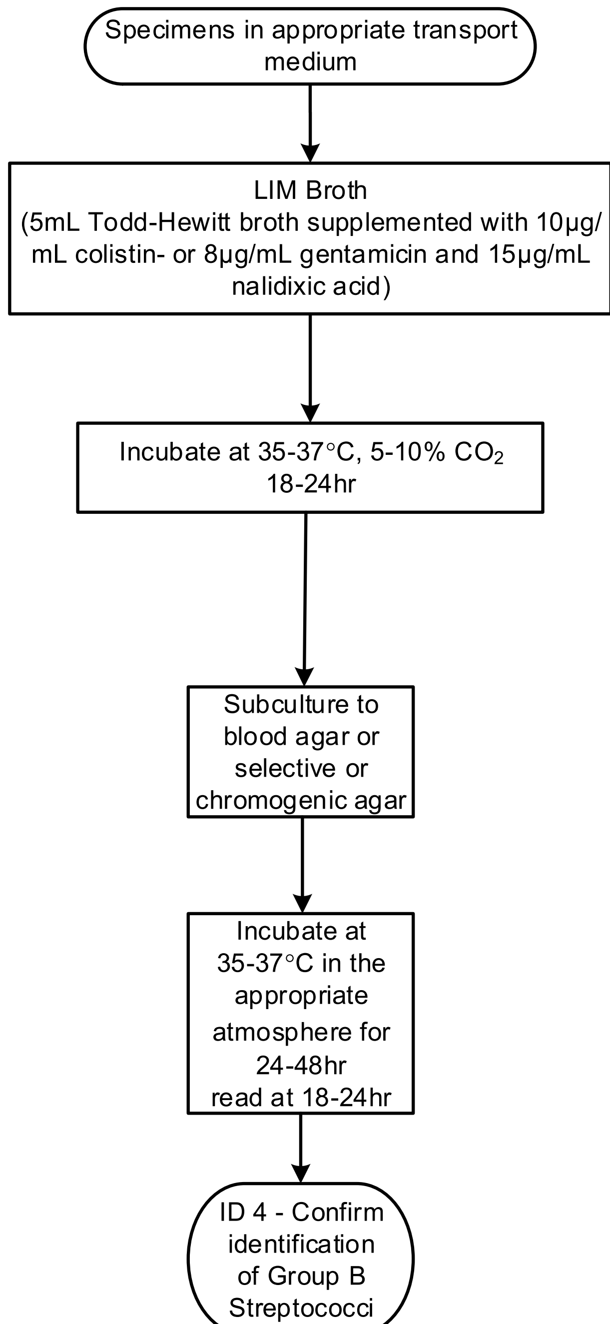
PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[51,52], Wales[53] and Northern Ireland[54].

Appendix: Detection of carriage of group B streptococci (S*treptococcus agalactiae*)



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Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 58 Detection of Carriage of Group B Streptococci

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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1st Consultation 18.08.14 – 22.09.14

Version of document consulted on – B 58do+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	19/09/2014	**Stakeholder Group**	Group B Strep Support
Section	Various		
Comment			

a. Page 7/19 – Colonisation.

What evidence has been used to support the change of carriage rates for the UK from up to 30% in the previous document to up to 20% in this? Daniels et al (2011 Intrapartum tests for GBS accuracy) found 21% (range 19-24%). Unpublished information provided to Group B Strep Support (GBSS) would suggest the 'up to 30%' statement is true for the UK.

b. Page 7/19 - Infection - Para 1

Suggest rewording the first sentence to say “Although GBS colonisation is not associated with disease in non-pregnant women, GBS can cause infection including bacteraemia, in pregnant women”. As the statement in your draft was written, it implies that pregnant women are not healthy. It is also important to include bacteraemia as well as infection in this statement.

c. Page 7/19 – Infection.

This section no longer includes the rate of GBS infection in babies. The previous version stated ... enhanced surveillance was undertaken in conjunction with the British Paediatric Surveillance Unit (London). The surveillance showed an incidence of 0.74 cases per 1000 live births and a mortality rate of 9.7%. The predominant GBS serotypes were III, Ia and V. This is important information and should be included.

d. Page 8/19 – Infection. Final paragraph in this section

2nd sentence: please change to say, “However, according to local protocols, women whose babies are judged clinically to be at high risk for the development of group B Streptococcal infection may be investigated for carriage.”

e. Page 8-9/19 - Method of Investigation.

If the statement “However, this enrichment broth is not totally selective for GBS, and other Gram positive cocci may be enriched by this method, possibly hiding GBS and leading to false negative results” is to be included, references are needed to support it, including the parameters of the likelihood of this happening and how the enriched culture method compares with a) the non-enriched culture method and b) the currently recommended risk based approach for false positive & false negative results.

f. Page 9/19 – Treatment.

The section on treatment has been removed and should be reinstated. This is important in putting the SMI in context. The text should be expanded to include

reference to the National Institute for Health and Care Excellence (NICE) Antibiotics for Neonatal Infection guideline which was published August 2012. g. Page 11/19 - Optimal Time and Method of Collection. The 5th paragraph refers to cultures being taken at 35-37 weeks of pregnancy. Although this is internationally recognised as the optimal time for samples to be taken for national screening programmes, some clinicians may wish to test women earlier, eg if the woman's at high risk of preterm labour, or indeed later. A statement needs to be included so that these groups are not excluded. h. Page 14/19 - Antimicrobial Susceptibility Testing. This section should be expanded to state that antimicrobial susceptibility testing is always necessary as there is growing problem of clindamycin resistance and clindamycin has been recommended for penicillin allergic patients. i. Page 16/19 - Appendix: Detecting method for Group B Streptococci Antimicrobial susceptibility testing has been left out of the flow-diagram. Please include it.
Evidence
Quoted in the above
Financial Barriers
None.
Health Benefits
Updating this SMI as described may help health professionals to improve the detection of group B Streptococcal carriage in pregnant women. Using this method, rather than the method described in UK SMI B 28 – *Investigation of genital tract and associated specimens* to investigate Group B Strep carriage, will enable significantly more accurate detection and thereby provide better information to the clinicians and the pregnant woman to inform her and her baby's care. Provided this method of testing is made available within the NHS for health professionals to access for pregnant women in their care, it will result in greater prevention of early-onset group B Strep infections in babies, which will save lives, prevent disability, reduce anxiety and reduce the costs associated with preventable group B Strep infection.

Recommended Action	a. **ACCEPT** The section has been amended with an additional reference. b. **ACCEPT** Amendments made. c. **ACCEPT** The incidence rate stated by The British Paediatric Surveillance Unit (BPSU) study has been re-inserted. d. **NONE** The paragraph has been removed. e. **NONE**

	The paragraph has been removed. f. **NONE** Recommendations on treatment is outside the scope of this SMI. g. **NONE** The sentence has been removed. h. **NONE** This is standard text for all SMIs. i. **NONE** It is not our practice to include susceptibility testing in the flowchart.

Comment Number	2		
Date Received	22/09/2014	**Professional Body**	UK National Screening Committee
Section	Pages 7-11		
Comment			

a. Scope [Page 7]

Acknowledgement of the screening recommendation is welcome. The aim of operating within the clinical guidance from the Royal College of Obstetricians and Gynaecologists (RCOG) and the National Institute for Health and Care Excellence (NICE) is appropriate for a document of this type and status. However the statement on 'clinical indications or when the test is requested' and recommendations later in the document are problematic in relation to this aim. It is also unclear how this SMI will be used in conjunction with other SMIs. If the changes we suggest are made, then the B28 - *Investigation of genital tract and associated specimens* would also have to be amended in order to align and present a consistent message.

b. Infection [page 7]

It would be useful to indicate the incidence of maternal and neonatal infection to put this into context; both are rare when the number of women who carry GBS is considered. An appropriate figure to cite would be from the British Paediatric Surveillance Unit surveillance study. The overall EOGBS rate of 0.48 / 1000 could be given along with the rate in term babies (~0.33 / 1000) to emphasise the point made about incidence rising with decreasing birth weight and prematurity.

c. "In addition stillbirths and premature delivery have also been attributed to GBS". [page 7]

This sentence should acknowledge that the association between GBS carriage and preterm birth is uncertain and is still debated.

d. Infection [page 8] "In the UK, routine antenatal screening……"

Current guidance from RCOG and NICE does not identify any clinical indications for which an Enrichment Culture Medium (ECM) test for GBS is recommended. The risk factors mentioned in the cited paragraph are addressed in these guidelines and have been discussed in another Public Health England document (ref 7) as candidate sub-populations for ECM testing. This document should provide a more prominent point of reference in the further consideration of the purpose of this SMI. Other risk groups for which an ECM test may be indicated should be identified and the evidence base for its use referenced.

The note about local protocols ignores national guidelines, which should take precedence in an SMI. The protocols are mentioned as exceptions to national guidance but are not referenced or explored in terms of the relevant risk groups or the evidence base.

Reference 4 is the UK National Screening Committee (UK NSC) review of screening and is not used appropriately at the end of the cited paragraph. The UK NSC document makes no recommendations regarding management of maternal risk factors. This should be replaced with reference to the RCOG and NICE guidelines. Both of these guidelines assume that 'known carriage' of GBS is from incidental detection arising from tests undertaken to explore vaginal or urinary tract infections. For consistency with the guidelines this should be emphasised.

e. Method of investigation [page 8] “Optimum yield will be achieved…..”

The CDC recommends screening for GBS carriage. This same strategy was considered by the UKNSC which recommended not to introduce screening in the UK.

Current prevention guidance from RCOG and NICE identified no clinical indication for testing for GBS carriage, therefore, the use of selective broth medium is not recommended. The only investigations recommended for infection and colonisations are not specific to a single bacterium, for example in cases of symptomatic presentation for severe or recurrent UTI. The non-selective media in this scenario would be a more appropriate choice as the cause of the UTI can be from a range of organisations of which GBS is one of the rarer types.

Therefore, there is no requirement for selective media to be used routinely for GBS specific investigation because there is no known indication or scenario outlined by RCOG/NICE where a clinician would ever just request GBS testing. As pointed out in the cited text, the preferential use of selective media will inhibit the sensitivity for other bacteria. This could be to the detriment of recommended investigations.

Finally, the selective media may be more sensitive in the detection of GBS colonisation when compared with non-selective media. However, there is no data to support the suggestion that the use of selective media is significantly better for the prediction neonatal infection, which should be the main outcome used to determine its value, furthermore the lower bacterial loads found using an ECM are thought to present a lower risk than the high bacterial loads that are more easily identified using non-selective media.

f. Rapid test assays [page 9] “However, the assays generally……”

This statement could be a little misleading, a number of studies have shown that the sensitivity of rapid tests has a detection rate of 70-90% compared to selective media cultures. It may be more appropriate to cite that United States Centers for Disease Control and Prevention (CDC) criteria for a good test (time required for

testing and 90% sensitivity/specificity threshold) is main reason why these methods aren't in common use.

g. Limitations of UK SMIs (page 9]

This section should provide references for sensitivity and specificity of selective media [it is not 100%] and the number of cases that transition between positive and negative (and visa versa) between delivery and 35-37 weeks.

Furthermore no studies have shown that selective media has an acceptable predictive value for neonatal infection, prediction of carriage at delivery has been the focus of studies of testing using the ECM. Two systematic reviews have been published on this (Health Technology Assessment, Health Technology Assessment Colbourn and Valkenburg).The predictive value for early onset disease would be much lower.

h. Selective Media in Screening Procedures [page 9]

GBS screening is not recommended. Furthermore, this use of selective media investigation for GBS is also not recommended within current prevention strategies. As it currently worded the statement does not fit with the SMI's aim of working within current guidance and recommendations.

i. Optimal Time and Method of Collection [Page 11] "At 35-37 weeks of gestation..."

This would imply that the test should be done to fit with a screening programme it is also noted elsewhere in the document that the sensitivity within a screening programme would be optimal at this time. It should be reinforced that there is no screening programme recommended in the UK and therefore this statement is out of place.

j. General comment

If the SMI is for GBS testing in clinically indicated circumstances and not for screening this is currently unclear. The circumstances in which the test might be used are not defined and explained within the document. The relationship of this SMI to others is not explained.

Without further information on these issues it is not possible to determine whether the SMI achieves its aim of operating within and supporting current guidance or whether the SMI contradicts current guidance.

It should also be noted that there is a forthcoming PHE position paper that will be published in the near future that will summarise, and provide evidence to support, the recommendations made to not offer ECM testing. When you look at both documents, it might appear that the two contradict one another "as both are PHE documents we would be eager to avoid any confusion that two publications might cause.

Evidence

Heath et al., 2004 Weisner et al., 2004 Schrag and Verani., 2012 Centre for Disease Control and Prevention Valkenburg et al., 2010 Colbourn et al., 2007 Group B Streptococcal Disease, Early Onset (Green-top 36) CG149 Antibiotics for early-onset neonatal infection: NICE guideline.

Financial Barriers

Yes, there is a potential policy clash with other PHE statements on GBS.

Health Benefits	
These issues have been addressed in the main body of comments.	
Recommended Action	a. **ACCEPT** The scope of the document has been amended to clarify the aims of the SMI. Associated SMIs will be updated to ensure consistency. b. **ACCEPT** The incidence rate stated by The British Paediatric Surveillance Unit (BPSU) study has been re-inserted. c. **NONE** The sentence has been removed. d. **NONE** The paragraph has been removed. e. **NONE** Outside the scope of SMI. f. **ACCEPT** The sentence has been amended. g. **NONE** This is a standard statement and is relevant to the SMI. h. **NONE** The scope of the document has been amended to clarify the aims of the SMI. i. **ACCEPT** The sentence had been removed. j. **NONE** It is the opinion of the working group that the scope of the amended SMI will be clear and that it does not contradict UK policy.

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 3			
Date Received	21/08/2014	**Lab Name**	Public Health Wales
Date Received	16/09/2014	**Professional Body**	HIS
Date Received	19/09/2014	**Lab Name**	Truro Microbiology

UK Standards for Microbiology Investigations

Detection of Enterobacteriaceae producing extended spectrum β-lactamases

NICE accredited
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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Issued by the Standards Unit, Microbiology Services, PHE

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UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

We also acknowledge Professor Neil Woodford for his considerable specialist input.

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Contents

Acknowledgments **2**

Amendment table **4**

UK SMI: scope and purpose **5**

Scope of document **8**

Introduction **8**

Technical information/limitations **14**

1 Safety considerations **16**

2 Specimen collection **16**

3 Specimen transport and storage **16**

4 Specimen processing/procedure **17**

5 Reporting procedure **21**

6 Notification to PHE, or equivalent in the devolved administrations **23**

Appendix: Flowchart for the screening and detection of ESBLs **25**

References **26**

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	6/17.08.16
Issue no. discarded.	4
Insert issue no.	5
Section(s) involved	**Amendment**
References.	GRADE information added to references.

Amendment no/date.	5/04.08.16
Issue no. discarded.	3
Insert issue no.	4
Section(s) involved	**Amendment**
Whole document.	B 59 formerly P 2 (previously QSOP 51). Title of document updated. Hyperlinks changed to gov.uk. Document presented in a bacteriology SMI template. The document gives recommendations on the testing of clinical or screening specimens for the detection of Enterobacteriaceae that produce an extended-spectrum β-lactamase (ESBL). Links to different websites/articles updated. Technical limitations/ information section updated.
Page 2.	Updated logos added.
Reporting procedure.	This has been updated with appropriate information.
Appendix.	Flowchart produced for easy guidance.
References.	References reviewed and updated.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

Public Health England. (2016). Detection of Enterobacteriaceae producing extended spectrum β-Lactamases. UK Standards for Microbiology Investigations. B 59 Issue 4.1. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

A50711295

Scope of document

Type of specimen

Screening specimens include stool, rectal or peri-rectal cultures. Clinical specimens include blood, wounds or urine.

Note: Ideally stool or rectal swabs should be submitted for screening.

This SMI describes the examination of clinical or screening specimens for the detection of Enterobacteriaceae that produce an extended-spectrum β-lactamase (ESBL).

This document should not be applied to isolates with carbapenem resistance – these may have ESBLs (or other enzymes) combined with porin loss, or may have acquired carbapenemases or may have both a carbapenemase and an ESBL. Further advice on detection of carbapenem-resistant isolates is provided in B 60 - Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases).

This SMI should be used in conjunction with other SMIs.

Introduction

The term "ESBL" is used in this document to mean acquired class A β-lactamases that hydrolyse and (usually) confer resistance to oxyimino- '2nd and 3rd generation' cephalosporins, eg cefuroxime, cefotaxime, ceftazidime and ceftriaxone, and 4th generation cephalosporins eg cefepime, cefpirome, but not cephamycins (eg cefoxitin) or carbapenems.

ESBLs include:

- cephalosporin-hydrolysing mutants of the TEM and SHV plasmid-mediated penicillinases of Enterobacteriaceae. These were the original ESBLs and over 400 such variants are known (see http://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase).
- CTX-M types. These evolved via the escape of chromosomal β-lactamase genes of *Kluyvera* species to plasmids. Over 170 variants are known, dividing into 5 major groups[1,2]
- minor types, eg VEB, PER and GES[3] – these are rare in Enterobacteriaceae and in the UK

ESBLs are not the only β-lactamases to confer resistance to cephalosporins while sparing carbapenems, but are the most important. Moreover, as plasmid-mediated enzymes, they have great potential for spread. They occur mostly in Enterobacteriaceae (eg *E. coli, Klebsiella* species and *Enterobacter* species). They should be distinguished from other modes of resistance to cephalosporins eg:

- derepressed chromosomal AmpC β-lactamases, especially in *Enterobacter* species
- plasmid-mediated AmpC β-lactamases eg CMY types, in *Klebsiella* species and *E. coli*
- hyperproduced K1 chromosomal β-lactamase in *K. oxytoca*

- advice on distinguishing all resistance mechanisms is available[4,5]

ESBLs are clinically important because they destroy cephalosporins that are used in the treatment of many severely ill patients. Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporins has been associated with increased mortality[6,7].

Until 2000 most ESBLs encountered in the UK were TEM and SHV mutants. They were largely seen in *K. pneumoniae*, including strains causing hospital outbreaks, but did not penetrate *E. coli* or community strains to any major extent. Since 2000, CTX-M ESBLs have proliferated. Unlike earlier types, these are often seen in *E. coli* from the hospital/community interface, eg from urinary infections among elderly out-patients with recent hospitalisation, those who are catheterised, and who have underlying disease [8]. Many patients with infections due to ESBL producers lack recent contact with hospitals; these may be admitted with serious secondary infections, eg bacteraemia where delays in effective therapy increase the risk of death[7].

Similar increases in ESBL prevalence, owing to dissemination of CTX-M enzymes have occurred also in Europe[8], Asia[9,10] and North America[11,12]: whilst CTX-M types have long been prevalent in Argentina[13]. The predominant CTX-M types vary with the country: CTX-M-15 dominates in most of Europe and Asia from India westwards, also North America[12,14]; CTX-M-2 in South America[13] and Israel[15]; CTX-M-14 in the Far East[10] and Spain[2]. The association with *E. coli* and greater community penetration persists irrespective of the particular enzyme. One *E. coli* lineage - Sequence Type (ST) 131- is an especially common ESBL host, especially for CTX-M-15 enzyme, and is disseminated internationally, including in the UK[16].

All cephalosporins except cephamycins (eg cefoxitin and cefotetan) are substrates for ESBLs, but resistance is not always high level, complicating detection and interpretation[4]. Many producers are multi-resistant to non-β-lactam antibiotics including quinolones, aminoglycosides and trimethoprim.

Laboratory detection: screening and confirmation

ESBLs may be detected fortuitously during the processing of clinical samples. Alternatively, they may be detected during targeted screening of faecal samples.

How to recognise ESBL Producers

There are several ways to recognise ESBL producers, as outlined in the main body of this document; the strategy below is the simplest way to meet these guidelines.

Enterobacteriaceae from hospitalised patients

- test both cefotaxime and ceftazidime on the first-line panel, or test cefpodoxime. Unless cefpodoxime is tested, it is required that both cefotaxime (or ceftriaxone) and ceftazidime are used as indicator cephalosporins, as there may be large differences in MICs of cefotaxime (or ceftriaxone) and ceftazidime for different ESBL-producing isolates. Cefpodoxime is the most sensitive individual indicator cephalosporin for detection of ESBL production. Cefpodoxime may be used for screening, but not for confirmation testing as it is less specific than the combination of cefotaxime (or ceftriaxone) and ceftazidime

- perform ESBL confirmatory tests (below) on isolates found resistant to any of cefotaxime, ceftazidime or cefpodoxime

Enterobacteriaceae from community patients

- test cefpodoxime as an indicator on first-line panel
- perform ESBL confirmatory tests (below) on isolates found resistant to cefpodoxime

Note: The spread of CTX-M enzymes into out-patient/community *E. coli* means that the indicator cephalosporin(s) should be tested first-line against all Enterobacteriaceae.

To confirm ESBL production in isolates found resistant to cefotaxime/ceftazidime or cefpodoxime

Use cefpodoxime/clavulanate combination discs for all Enterobacteriaceae except *Enterobacter* species and *Citrobacter freundii,* where cefpirome/clavulanate or cefepime/clavulanate combination discs are used.

Note:

Identification to genus/species level is highly desirable for the interpretation of resistance patterns. As a minimum, identification should be undertaken on all isolates found resistant to cefotaxime, ceftazidime or cefpodoxime.

The basic strategy to detect ESBL producers, outlined above, is to use an indicator cephalosporin to screen for likely producers, then to seek cephalosporin/clavulanate synergy, which distinguishes ESBL producers from strains that hyperproduce either AmpC or K1 enzymes[4].

Screening

The ideal indicator cephalosporin is one to which all ESBLs confer resistance, even when production is scanty[4]. Choice is predicated by the following general traits:

- TEM and SHV ESBLs – obvious resistance to ceftazidime, variable to cefotaxime
- CTX-M ESBLs – obvious resistance to cefotaxime, variable to ceftazidime
- all ESBLs – resistance to cefpodoxime, however, low-level cefpodoxime resistance is common in isolates with no ESBL or other substantive mechanism[17]
- cefuroxime, cephalexin and cephradine are unreliable indicators for ESBL production and are not recommended.

Selective culture media

Chromogenic media have been developed for detection of ESBL-producers in faecal screening whereas for routine diagnostic testing using EUCAST disc diffusion method, the screening cut off values could be employed for detecting ESBLs (see EUCAST detection of resistance mechanisms) as referred to in section 4.7 of this document[18-20]. Although chromogenic agar use may have its limitation, such as being likely to be less specific, particularly in areas where ESBL producers are commonplace, they are still the preferred option[19-24]. Some commercial products include selective antimicrobial

agents incorporated into the medium. Others will require placement of indicator cephalosporin discs.

Clinical specimens can also be screened using MacConkey or CLED agar with an antibiotic disc. Although not a well validated or tried method, if used by laboratories, they should ensure that this has been validated and verified locally.

For further information on the different screening methods, see section 4.7.

Confirmatory tests for ESBLs: inhibitor-based tests

Enterobacteriaceae isolates resistant to any indicator cephalosporin but susceptible to carbapenems in the screening tests above should be subjected to confirmatory tests. These depend on demonstrating synergy between clavulanate and the indicator cephalosporin(s) to which the isolate was found resistant. Three methods can be used:

- **Double disc synergy tests**

  A plate is inoculated with the test organism as for a routine susceptibility test. Discs containing cefotaxime and ceftazidime 30µg (or cefpodoxime 10µg) are applied either side of one with co-amoxiclav 20+10µg; and are placed 20mm away (centre to centre) from it. This distance is optimal for cephalosporin 30µg discs[4]. However, it has been suggested that the sensitivity of this test can be increased by reducing the distance between the discs to 15mm or expanding to 30mm for strains with very high or low levels of resistance respectively[25].

  ESBL production is inferred when the zone of either cephalosporin is expanded by the clavulanate. The method is inexpensive, but the optimal disc separation varies with the strain and some producers may be missed. It is therefore not recommended.

Figure 1: Detection of ESBL production using the double disc method.
The disc on the left is cefotaxime (30µg): the disc in the centre is co-amoxiclav (20+10 µg): the disc on the right is ceftazidime (30µg). Note the expansion of the zones around the cefotaxime and ceftazidime discs adjacent to the co-amoxiclav (courtesy of Jenny Andrews of the Sandwell and West Birmingham NHS Trust[26]).

- **Combination disc tests[27,28]**

  These compare the zones of cephalosporin discs to those of the same cephalosporin plus clavulanate. These are commercially available. According to the supplier, either the difference in zone diameters, or the ratio of diameters, is compared, with zone diameter increases of ≥5mm or ≥ 50 % in the presence of the clavulanate implying ESBL production[28,29]. These tests are inexpensive and do not require critical disc spacing, but care should be taken regarding controls (see below) especially if the discs are from different batches.

- **Gradient ESBL strips**

  These have a cephalosporin gradient at one end and a cephalosporin plus clavulanate gradient at the other. Users should follow the manufacturer's instructions, including for a heavier inoculum than in BSAC disc tests. ESBL production is inferred if the MIC ratio for cephalosporin alone compared with cephalosporin + clavulanate MIC is ≥8. These are accurate and precise, but more expensive than combination discs. The test should be used for confirmation of ESBL production only and is not reliable for determination of the MIC.

- **Automated systems**

  There are many commercially available systems for ESBL detection. Although some authors report false positives, automated or semi-automated systems generally can be used to detect ESBLs[30-33]. Some cards and panels include cephalosporin-clavulanate synergy tests; others infer ESBL production from overall antibiograms. Care should be taken to ensure that control strains (see below) give the appropriate result with the card or panel used, as problems have arisen with particular card types[34].

Confirmatory tests for ESBLs: rapid methods

Molecular tests: PCR has been successfully utilized for the detection of ESBL genes directly from clinical or screening samples[35]. Obvious advantages include a greater speed of detection and potentially a higher sensitivity than that offered by culture[19]. Disadvantages include a higher cost for processing samples and the need for specialised equipment and/or expertise and so might be considered expensive in some settings.

Gene sequencing and DNA microarray-based method have also been recommended for the genotypic confirmation of the presence of the ESBL genes[36,37]. Test results are usually obtained within 24hrs, however, molecular methods may not detect sporadically occurring ESBL genes or new ESBL genes[25].

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-TOF): This is increasingly available to diagnostic laboratories; and has definite potential to discriminate antibiotic-resistant strains due to ESBL and carbapenemase production from non-producing strains, but this performance is not yet sufficiently reliable for routine microbiological diagnostics[38]. However, MALDI-TOF has been shown to be a rapid and efficient method for the early detection of ESBL-producing Enterobacteriaceae from clinical samples such as positive blood cultures thus allowing early administration of an appropriate antibiotic therapy[39].

This assay has also been noted to be much faster than the methods used routinely in clinical practice. It has the potential to provide an answer on day 1 if used with a clinical specimen or on day 2 if used on colonies. This option is not commercially available at the time. The overall expected time from the protein extraction to the spectrum acquisition and analysis is <2hr. Another additional advantage is its relatively low cost[39].

Controls for ESBL tests

Quality Control of the cephalosporin discs used in the routine primary screening should follow standard EUCAST/BSAC or CLSI recommendations.

Positive controls should be used to ensure the performance of ESBL confirmatory tests. Three ESBL-positive *E. coli* strains suitable for purpose are available from the NCTC (www.phe-culturecollections.org.uk/media/63614/m01520130827v4_antimicrobresmech-a4.pdf). They are as follows:

- CTX-M-15 (cefotaximase, less active against ceftazidime) NCTC 13353
- TEM-3 (broad-spectrum ESBL) NCTC 13351
- TEM-10 (ceftazidimase, less active against cefotaxime) NCTC 13352

Alternatively, some strains may be obtained commercially from other suppliers.

Table 2 showing ESBL control strains available from the NCTC

2. Extended-Spectrum β-Lactamases (ESBL):

2.1 TEM β-lactamases

Organism	NCTC® Strain Reference	Characteristics
Escherichia coli	NCTC 13351	TEM-3 ESBL – Transconjugant of strain isolated in Clermont Ferrand in 1985
Escherichia coli	NCTC 13352	TEM-10 ESBL – Transconjugant of original TEM-10 producer isolated in Chicago in 1988

2.2 SHV β-lactamases

Organism	NCTC® Strain Reference	Characteristics
Klebsiella pneumoniae	NCTC 13368	SHV-18 (ATCC 700603)

2.3 CTX-M β-lactamases

Organism	NCTC® Strain Reference	Characteristics
Escherichia coli	NCTC 13353	Strain EO 487. CTX-M-15 ESBL producer. Control strain for group 1 bla_{CTX-M} multiplex PCR assays
Escherichia coli	NCTC 13441	Strain EO 499. CTX-M-15 ESBL producer – Uropathogenic strain O25:H4 sequence type (ST) 131. Clinical isolate harbouring sequenced plasmid pEK499 (see NCTC 13400); Control strain for group 1 bla_{CTX-M} multiplex PCR assays
Escherichia coli	NCTC 13400	Strain Tr499 = DH5-α derivative. Source of pEK499 (fully sequenced plasmid GenBank Accession No EU935739) encoding CTX-M-15 enzyme. Fusion of type FII and FIA replicons, and harbours 10 antibiotic resistance

The CLSI recommends *K. pneumoniae* ATCC 700603 as a single ESBL-producing QC control. This strain may be sourced from the ATCC.

Either *E. coli* NCTC 10418 or ATCC 25922 should also be used as a negative control in ESBL confirmation tests. Negative controls are especially important when cephalosporin and cephalosporin plus clavulanate combination discs are from different batches, which may vary in retained potency. Zones of the cephalosporin and cephalosporin and clavulanate discs for ESBL-negative *E. coli* should be equal or within 2mm. Any greater difference implies malfunction or deterioration.

Detecting ESBLs in AmpC-Inducible species

ESBLs are harder to detect in species of *Enterobacteriaceae* with inducible, chromosomal AmpC enzymes (eg *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* and *Serratia*) than in *E. coli* and *Klebsiella* because AmpC activity induced by the clavulanate may attack the indicator cephalosporin, masking any synergy arising from inhibition of the ESBL.

- if ESBL tests are to be done on AmpC-inducible species it is best to use an AmpC-stable cephalosporin (ie cefepime or cefpirome) in the clavulanate synergy tests[40]. Cefepime-clavulanate gradient strips or combination discs and cefpirome-clavulanate combination discs are available. Once again, a >8-fold MIC reduction or >5mm zone expansion indicates a positive ESBL result
- cephalosporins are in any case not recommended as therapy for infections due to AmpC-inducible species, owing to the risk of selecting AmpC-derepressed mutants, with consequent failure[41]
- ESBL tests have poor sensitivity (but good specificity) for *Enterobacter* species even if using cefepime or cefpirome, especially if AmpC is concurrently hyperproduced. Some producers are only revealed by molecular testing

Distinguishing ESBLs from carbapenemases

The presence of ESBLs may also be masked by carbapenemases such as MBLs or KPCs (but not OXA-48-like enzymes) and/or severe permeability defects (refer to B 60 -Screening and detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)). The epidemiological importance of ESBLs in these contexts could be questioned, since the carbapenemase has greater public health importance, but if detection is still considered relevant it is recommended that molecular methods for ESBL detection are used.

Distinguishing ESBLs from K1 enzyme

Around 10-20% of *K. oxytoca* isolates hyperproduce their class A “K1”chromosomal β-lactamase. These are resistant to cefpodoxime, aztreonam and piperacillin-tazobactam, but not ceftazidime[5].

- they may give weak positive clavulanate synergy tests with cefotaxime or cefepime (not ceftazidime), leading to confusion with ESBL producers[42]. K1 hyperproduction should be suspected if a *Klebsiella* isolate is indole-positive and has high-level resistance to piperacillin/tazobactam, cefuroxime and aztreonam - but only borderline resistance or susceptibility to cefotaxime and full susceptibility to ceftazidime

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use,

laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[43,44]

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Quality control

The discs that are used should be quality control tested using disc diffusion methods and quality control strains as described in the BSAC or EUCAST or CLSI guideline documents. Follow guidelines for frequency of disc quality control testing and corrective action if results are out of range.

Chromogenic media

Chromogenic media are affected by light and plates should be stored in the dark and not left in the light before or after inoculation. Incubation times for chromogenic media should be as recommended by the manufacturers.

1 Safety considerations[43-59]

1.1 Specimen collection, transport and storage[43-48]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[43-59]

Containment Level 2 pathogens.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[51].

Refer to current guidance on the safe handling of all organisms documented in this SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Screening specimens include stool, rectal or peri-rectal cultures. Clinical specimens include blood, wounds or urine.

2.2 Optimal time and method of collection[60]

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible[60].

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium[61-65]

Collect specimens into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens[60]

There should be visible faecal material on the rectal or peri-rectal swabs taken.

Numbers and frequency of specimen collection are dependent on the clinical condition of patient or for screening specimens, on local policies and practices.

3 Specimen transport and storage[43,44]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[60].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[60].

4 Specimen processing/procedure[43,44]

4.1 Test selection

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

N/A

4.4.2 Supplementary / preparation of smears

N/A

4.5 Culture and investigation

Direct culture

Inoculate culture media with swab or other sample (refer to Q 5 – Inoculation of culture media in bacteriology).

Enrichment culture

Remove the cap aseptically from the container and place the swab(s) in the broth, break off (or cut) the swab-stick(s) and replace the cap.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Clinical samples submitted for diagnostic culture and susceptibility testing: Any condition + detection of ESBL-producing Enterobacteriaceae	Any sample	Process as requested in accordance with the relevant SOPS. Include the indicator antimicrobials: screening indicator drugs as per standardised method used and if using EUCAST, refer to Table 3 for the EUCAST cut off values on any Enterobacteriaceae isolated	35-37	Aerobic	18-24hr	≥18hr	ESBL producing Enterobacteriaceae
Screening: Screening test for ESBL-producing Enterobacteriaceae	Screening specimens – Stool, Rectal or Peri-rectal swabs	Chromogenic agar using 30µg cefotaxime and ceftazidime 30µg (or 10µg cefpodoxime only)[66] OR alternatively, MacConkey[67]/ CLED agar + 30µg cefotaxime and 30µg ceftazidime (or 10µg cefpodoxime only)	35-37	Aerobic	18-24hr	≥18hr	ESBL producing Enterobacteriaceae most especially *Klebsiella* species *Escherichia coli*
Other organisms for consideration – ESBLs are harder to detect in species of Enterobacteriaceae with inducible, chromosomal AmpC enzymes (eg *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* and *Serratia*) but some confirmatory tests (cefpirome/clavulanate or cefepime/clavulanate) can be used for identification of these. Refer to the Introduction.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Klebsiella species *Escherichia* species	species level ID 16 - Identification of Enterobacteriaceae

Enterobacter species *Citrobacter* species	
Pseudomonas species *Acinetobacter* species *Stenotrophomonas maltophilia*	species level ID 17 - Identification of *Pseudomonas* species and other non-glucose fermenters **Note:** The methods described herein are not suitable for detecting ESBLs in *Acinetobacter* species, which are often susceptible to clavulanic acid and so may yield a false ESBL-positive result. Ceftazidime-clavulanate synergy may be used to indicate ESBL production (usually VEB or PER enzymes) in isolates of *Pseudomonas* species, but this is uncommon in the genus and should not be routinely sought.

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial susceptibility testing

Testing cultured bacterial isolates

The recommended methods for detecting Enterobacteriaceae for ESBL production in routine samples are broth dilution, agar dilution, disc diffusion or an automated system.

The indicator drugs should be included in primary susceptibility testing done eg by the method of the British Society for Antimicrobial Chemotherapy (http://bsac.org.uk/susceptibility/methodologylatestversion/)[68,69]. Refer to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)/ British Society for Antimicrobial Chemotherapy (BSAC), or Clinical and Laboratory Standards Institute (CLSI) guidelines. Species identification is highly desirable to allow proper interpretation of results. BSAC recommended breakpoints for the cephalosporins advocated are updated annually and should be sought from the link above.

Table 3: ESBL detection criteria for Enterobacteriaceae[25]

Method	Antibiotic	Perform ESBL-testing if
Broth or agar dilution [1]	Cefotaxime/ceftriaxone AND Ceftazidime	MIC >1 mg/L for either agent
	Cefpodoxime	MIC >1 mg/L
Disc diffusion [1]	Cefotaxime (5µg) / Ceftriaxone (30µg) AND Ceftazidime (10µg)	Inhibition zone < 21 mm Inhibition zone < 23 mm Inhibition zone < 22 mm
	Cefpodoxime (10µg)	Inhibition zone < 21 mm

[1] With all methods either test cefotaxime or ceftriaxone AND ceftazidime OR cefpodoxime can be tested alone.

Note: It should be noted that the inhibition zone sizes in Table 3 apply only when the standardised methodology (EUCAST/ BSAC or CLSI) is used and not on MacConkey/CLED agar plates.

Direct testing of clinical or screening samples with indicator discs

In clinical or screening samples inoculated directly on agar plates with cephalosporin indicator discs, any isolates of presumptive Enterobacteriaceae with a zone size of within 20mm should be identified and submitted for formal susceptibility testing in accordance with EUCAST/ BSAC, or CLSI methodology[25,68].

Confirmatory Tests for ESBLs: inhibitor-based tests

Enterobacteriaceae isolates resistant to any indicator cephalosporin, but susceptible to all carbapenems in the screening tests above, should be subjected to confirmatory tests. These depend on demonstrating synergy between clavulanate and the indicator cephalosporin(s) to which the isolate was found resistant.

Table 4: ESBL confirmation methods for Enterobacteriaceae that are positive in the ESBL screening[25]

Method	Antimicrobial agent (disc content)	ESBL confirmation is positive if
ESBL gradient test	Cefotaxime +/- clavulanic acid	MIC ratio ≥ 8 or deformed ellipse Present
	Ceftazidime +/- clavulanic acid	MIC ratio ≥ 8 or deformed ellipse Present
Combination disc diffusion test	Cefotaxime (30 µg) +/- clavulanic acid (10 µg)	≥ 5 mm increase in inhibition zone
	Ceftazidime (30 µg) +/- clavulanic acid (10 µg)	≥ 5 mm increase in inhibition zone
Double disc synergy test	Cefotaxime, ceftazidime and cefepime	Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disc
Other organisms for consideration – ESBLs are harder to detect in species of Enterobacteriaceae with inducible, chromosomal AmpC enzymes (eg *Enterobacter*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* and *Serratia*) but some confirmatory tests (cefpirome/clavulanate or cefepime/clavulanate) can be used for identification of these. Refer to the Introduction.		

4.8 Referral for outbreak investigations

In England, the AMRHAI Reference Unit at PHE Colindale does not seek to confirm all ESBL producers, but the following should be submitted:

- representative isolates from major outbreaks
- representative isolates from unusual settings, eg neonatal units, especially if multiple cases occur

- isolates giving concerns based on a patient's history (contact laboratory to discuss)

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit
Bacteriology Reference Department
National Infections Service
Public Health England
61 Colindale Avenue
London
NW9 5EQ

https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services

Telephone: +44 (0) 208 3276511/ 7877

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Wales

http://www.wales.nhs.uk/sites3/page.cfm?orgid=457&pid=28768

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

N/A

5.1.1 Microscopy reporting time

N/A

5.2 Culture

Screening samples

Negatives

"ESBL-producing Enterobacteriaceae not isolated"

Positives

"ESBL-producing Enterobacteriaceae (insert genus and species identification) isolated" eg ESBL-producing *Klebsiella pneumoniae* isolated

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated (noting the caveats below). Prudent use of antimicrobials according to local and national protocols is recommended.

5.3.1 Cephalosporins

There is a division of opinion about the reporting of cephalosporin susceptibility for ESBL producers. For several years it was considered, by BSAC/EUCAST and CLSI and based on clinical experience that all ESBL producers should be reported as resistant to all cephalosporins and aztreonam, irrespective of susceptibility test results.

Latterly, EUCAST and CLSI have taken the contrary view, arguing that, with the low breakpoints now adopted by both organisations, cephalosporin susceptibility results can be taken at face value, and that cephalosporins can be used as therapy so long as ESBL producers appear susceptible *in vitro*[70]. This view is based upon pharmacodynamic analysis, animal studies and on several reports of positive treatment outcomes when MICs were 1-2mg/L.

However, this revised view is challenged on the grounds (i) that the evidence of predictable clinical success for cephalosporins against low-MIC ESBL producers is far from overwhelming, with cephalosporin failures also reported *vs.* low-MIC ESBL-positive strains, and (ii) 'susceptible' MIC and zone test results for ESBL producers often have poor reproducibility[37].

In the face of this disagreement, the best advice is to apply utmost caution if cephalosporins are to be used in severe infections due to ESBL producers.

It should also be added that the great majority of ESBL producers in the UK are clearly resistant to all oxyimino-cephalosporins at BSAC-EUCAST breakpoints and that this debate relates only to a minority of isolates (this situation is different in countries where producers of CTX-M-2 and -14 dominate, as MICs of ceftazidime for these often are 2-4mg/L).

Combinations of a cephalosporin with co-amoxiclav should be effective in principle, but have not been formally evaluated and may be antagonistic against some ESBL-negative *Enterobacter* species[71].

5.3.2 Penicillins and penicillin-inhibitor combinations

Organisms with ESBLs are resistant to all parenteral penicillins except temocillin, which is stable and generally active. Mecillinam may appear active *in vitro*, but its efficacy remains unproven, with anecdotal reports of failures as well as one positive case series[72,73].

Susceptibility to β-lactamase inhibitor combinations varies with the isolate. ESBLs are inhibited by tazobactam and clavulanate but many isolates with CTX-M-15 (the commonest ESBL in the UK) also have OXA-1, an inhibitor-resistant penicillinase, conferring resistance.

A recent analysis showed that inhibitor combinations can be used against ESBL producers when these appear susceptible *in vitro*[74].

5.3.3 Carbapenems

Carbapenems (imipenem, ertapenem, meropenem and doripenem) are stable to ESBLs and remain active against ESBL producers unless the organism

- also loses porins, reducing permeability - a mechanism that particularly compromises ertapenem or
- acquires DNA encoding a carbapenemase[75]. For further information, refer to B 60 – Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)

6 Notification to PHE[76,77], or equivalent in the devolved administrations[78-81]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

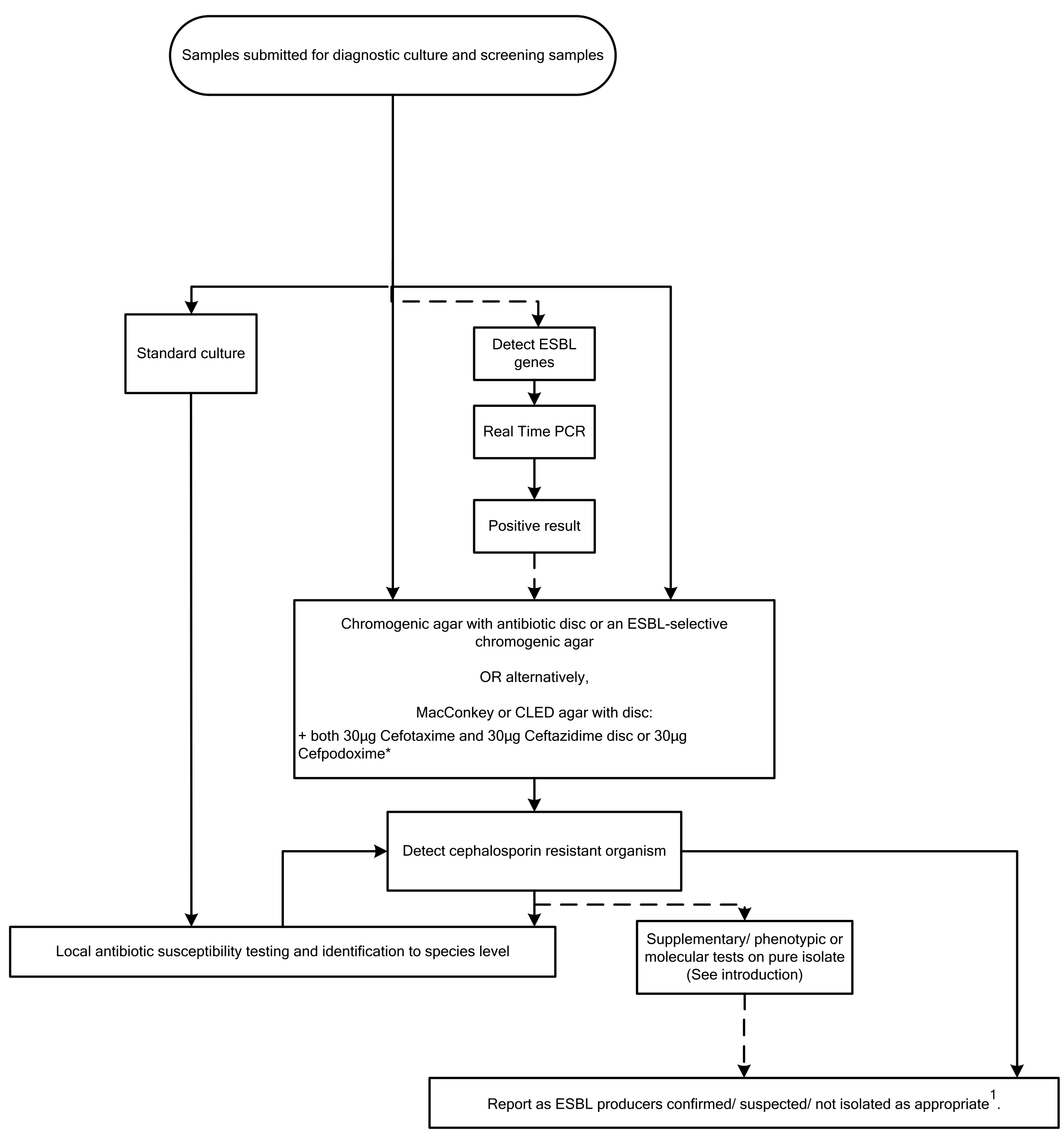
Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare

Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[78,79], Wales[80] and Northern Ireland[81].

Appendix: Flowchart for the screening and detection of ESBLs[25]



[1] If concerned about a result based on a patient's history, send to the PHE reference laboratory for further testing.

Note: The branch with the dotted lines in this flowchart is optional but useful for diagnostic laboratories that have molecular methods available locally. For more information, see link: http://www.eucast.org/resistance_mechanisms/.

The flowchart is for guidance only.

References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, eg case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

1. Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. AntimicrobAgents Chemother 2004;48:1-14. **B, III**

2. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G et al. CTX-M: changing the face of ESBLs in Europe. J AntimicrobChemother 2007;59:165-74. **C, III**

3. Akinci E, Vahaboglu H. Minor extended-spectrum beta-lactamases. ExpertRev AntiInfect Ther 2010;8:1251-8. **B, III**

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7. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J AntimicrobChemother 2007;60:913-20. **C, III**

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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 59 Detection of enterobacteriaceae producing extended spectrum β-lactamases

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Version of document consulted on: B 59dzb+

Proposal for changes

Comment number	1		
Date received	15/07/2015	**Lab name**	Medical Microbiology Northern Trust
Section	Introduction and How to recognise ESBL producers		
Comment			
Introduction a. They should be distinguished from other modes of resistance to cephalosporins and non-metallo (KPC and OXA-48) carbapenemases. I think OXA-48 should be removed as OXA-48 by itself does not lead to cephalosporin resistance. How to recognise ESBL producers Title: b. How to recognise ESBL Pproducers change to Producers c. In Enterobacteriaceae from hospitalised patients • Pperform ESBL confirmatory Tests change to Perform			
Financial barriers			
No experience.			
Health benefits			
None.			
Recommended action	a. **ACCEPT** This has been updated in the document. b. **ACCEPT** This has been updated in the document. c. **ACCEPT** This has been updated in the document.		

Respondents indicating they were happy with the contents of the document

Overall number of comments: 2			
Date received	09/07/2015	**Professional body**	IBMS
Date received	10/07/2015	**Lab name**	NHS Lanarkshire

UK Standards for Microbiology Investigations

Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)

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This publication was created by Public Health England (PHE) in partnership with the NHS.

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Bacteriology | B 60 | Issue no: 3.1 | Issue date: 01.06.2022 | Page: 1 of 32

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Contents

Acknowledgments **2**

Amendment table **4**

1. General information **6**

2. Scientific information **6**

3. Scope of document **6**

4. Background **6**

5. Safety considerations **15**

6. Investigation **16**

7. Antimicrobial susceptibility testing **19**

8. Referral to reference laboratories **24**

Appendix 1: Flowchart for the detection of carbapenemases on cultured isolates and screening samples **26**

References **27**

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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	5/01.06.2022
Issue number discarded	3
Insert issue number	3.1
Anticipated next review date*	18.09.2023
Section(s) involved	**Amendment**
Section 8: Referral to reference laboratories	Replaced 'Anaerobic reference unit (ARU) Cardiff' with 'Specialist Antimicrobial Chemotherapy Unit'.

Amendment number/date	4/18.09.2020
Issue number discarded	2.1
Insert issue number	3
Anticipated next review date*	18.09.2023
Section(s) involved	**Amendment**
Whole document	Document has been transferred to a new template. Clarity on different settings when testing for carbapenemase producers added to the document. Headings changed. Clarification of use of selective media with carbapenem disc if chromogenic agar is not available. Whole document restructured for clarity
Section 6. Investigation	Investigation table amended. Updated to include new regulations regarding reporting of acquired carbapenemase-producing Gram-negative bacteria identified in human samples to PHE. Interpreting and reporting results for culture amended.

Section 7.2.1 Controls for carbapenemase tests	Control strains producing carbapenemases available from the NCTC amended.
Appendix 1	Flowchart for the detection of carbapenemases on cultured isolates and screening samples amended.
Appendix	Appendix 2 removed. Readers are referred to detailed evaluations published to date.
References	References reviewed and updated.

*Reviews can be extended up to five years subject to resources available.

1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

The UK SMI gives recommendations on screening and detection of acquired 'carbapenemases' (carbapenem-hydrolysing β-lactamases). It should be used in conjunction with any local documents and PHE's framework of actions to contain carbapenemase-producing Enterobacterales.

This UK SMI should be used in conjunction with other relevant UK SMIs.

This document is intended to refer to different settings when testing for carbapenemase producers:

- Setting 1: screening of clinical samples for the presence of carbapenem resistance in particular carbapenemases
- Setting 2: preliminary detection of carbapenem resistance in cultured isolates from clinical samples.
- Formal confirmation of carbapenem resistance by susceptibility testing of suspected isolates.

We are following EUCAST recommendations for Setting 2 and formal confirmation of carbapenem resistance. There are no agreed international recommendations in place for Setting 1.

4. Background

The term 'carbapenemase' is used to mean any β-lactamase that hydrolyses carbapenems. Carbapenems (any or all of doripenem, ertapenem, imipenem and meropenem) are antimicrobials of last resort and are crucial for preventing and treating life-threatening nosocomial infections. Carbapenemases are clinically important because they destroy, and so may confer resistance to, carbapenems (and usually most other β-lactams). Carbapenemases are found naturally in a few clinically relevant bacteria, such as *Stenotrophomonas maltophilia*, *Aeromonas* species, and 'chryseobacteria', including *Elizabethkingia meningoseptica*[1]. *Acinetobacter baumannii* also has the gene for an intrinsic carbapenemase (OXA-51-like), but this confers reduced susceptibility or resistance to carbapenems only when its expression is up-regulated by genetic reorganisation[2].

In addition, non-susceptibility or resistance to specific carbapenems is an intrinsic characteristic of some Gram-negative bacteria: most non-fermenters are naturally resistant to ertapenem (but not to other carbapenems); *Serratia* species and Proteeae have intrinsic poor susceptibility or low-level resistance to imipenem (but not to other carbapenems).

Studies have shown that *Bacteroides fragilis* can harbour a chromosomally mediated metallo-β-lactamase gene called cfiA (ccrA) which confers resistance to carbapenem[3,4].

This document focuses on acquired carbapenemases. Accurate identification of bacteria to genus or species level will allow laboratories to recognise the producers of intrinsic carbapenemases detailed above.

4.1 Acquired carbapenemases

Acquired carbapenemases are diverse and include three of four Ambler's molecular classes of β-lactamases[5,6].They are detailed below:

Class A enzymes: All hydrolyse carbapenems effectively and are partially inhibited by clavulanic acid. The most widespread carbapenemases in this class are the KPC enzymes; other, less frequently-encountered class A carbapenemases include some GES types (notably GES-5), IMI/NMC-A (in *Enterobacter* species), FRI (in *Enterobacter* species) and SME (in *Serratia marcescens*)[7]. KPC enzymes have been recorded in *A. baumannii* in Central America, and in *P. aeruginosa* in Central and South America, USA, China, the Caribbean, and occasionally in the UK[8-12 13,14].

Class B enzymes: Also known as 'metallo-β-lactamases' (MBLs) or 'metallo-carbapenemases'[5,6]. These differ fundamentally from all other β-lactamases because they require zinc ions in their active sites for activity[15]. Consequently, they are inactivated by metal ion chelators, such as EDTA[16]. The major MBL families encountered in the UK are the NDM, VIM and, less commonly, IMP types. Other types include GIM, SIM, DIM and SPM-1 enzymes, which have been found at a very low frequency in *P. aeruginosa* and *Citrobacter freundii* within the UK.

Class D enzymes: This class comprises many (>400) diverse β-lactamases, a few of which are carbapenemases[5,6,17]. Important carbapenemases within the family include OXA-23, -40, -51, -58 and their variants from *Acinetobacter* species and OXA-48-like enzymes in Enterobacterales; other rarer carbapenem-hydrolysing class D types include OXA-198 in *Pseudomonas* species.

Although some are chromosomally encoded (e.g. NMC-A/IMI and SME), many acquired carbapenemases are plasmid-mediated (especially when found in Enterobacterales), giving potential for spread between strains, species and genera.

Table 1. Carbapenemases currently known to be circulating in the UK by classification, activity and organisms

Enzyme type	Classification by ambler class	Activity spectrum	Organism(s)
KPC	A	All β-lactams	Enterobacterales; rare in *P. aeruginosa*
SME	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*S. marcescens*
NMC–A IMI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*Enterobacter* species; rare in other Enterobacterales
GES	A	Depends on enzyme variant. Some are ESBLs, others e.g. GES-5 are carbapenemases	*P. aeruginosa* and Enterobacterales
FRI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*Enterobacter* species

IMP **VIM** **NDM** GIM, DIM, SIM, SPM-1 (infrequently identified in the UK)	B (metallo-β-lactamases)	All β-lactams except monobactams (aztreonam)	*Pseudomonas* species; *Acinetobacter* species; Enterobacterales
OXA	D	Carbapenems (note that many OXA enzymes are NOT carbapenemases)	*A. baumannii*; Enterobacterales and rare in *P. aeruginosa*

Note: The enzyme types in bold are the five main carbapenemase families found in the UK, the so-called 'big five'.

Delayed recognition and inappropriate treatment of severe infections caused by carbapenemase producers is associated with increased mortality[18]. Many producers are multi-resistant to non-β-lactam antibiotics including quinolones and aminoglycosides.

The carbapenem MIC ranges for Enterobacterales producing each of the 'big five' carbapenemases (KPC, OXA-48-like, NDM, VIM and IMP) span from below the susceptible clinical breakpoints to high-level resistance, and when combined with the diversity of carbapenemase types, this means that few, if any, strategies reliably detect all carbapenemase producers. Nevertheless, the carbapenem MICs of most carbapenemase-producing bacteria will be above the epidemiological cut-off (ECOFF) values defined by EUCAST even if some isolates are not clinically resistant (that is, MICs remain equal to or below the clinical breakpoints). ECOFF values (or breakpoints) mark the limit of the wild-type population by a statistical definition, and isolates with higher MICs/lower zone diameters represent non-wild-type isolates.

The level of carbapenem resistance displayed by some carbapenemase producers is a cause for concern. Higher MICs are observed when carbapenemase producers also lack major porins. Among strains with lower MICs and without porin loss there is potential for carbapenemase producers to spread undetected. This concern is greatest with OXA-48-like enzymes in Enterobacterales, which can give very low level carbapenem resistance[19], without cross-resistance to cephalosporins. KPC enzymes and MBLs tend to confer broader effects on the β-lactam resistance profile of the host strain.

4.2 Complexities of detection of carbapenemase production

Clinical laboratories should have a high index of suspicion while investigating isolates for carbapenemase production and should be alert to two confounders:

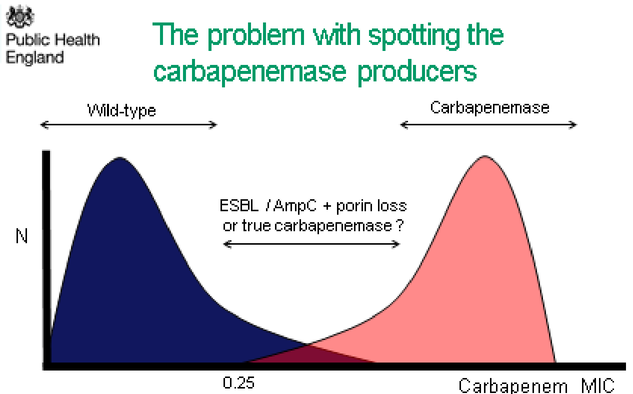
a) not all carbapenem-resistant isolates produce a carbapenemase (resistance can be mediated by other mechanisms, such as the combination of ESBL/AmpC plus impermeability, as below),

b) not all carbapenemase producers are resistant to carbapenems

Carbapenemases are not the only mechanism of acquired resistance to carbapenems but are the most important from a public health perspective. Other mechanisms include the following:

- Enterobacterales with ESBL or AmpC enzymes may lose outer membrane porins (through mutations or other disruptions in chromosomal genes), reducing carbapenem uptake[20]. In contrast to carbapenemases, these combinatorial mechanisms of carbapenem resistance are not transferable between strains (though the contributing ESBL might be) and the porin-deficient mutants may have reduced fitness and be less likely to spread in healthcare settings. This mechanism is seen most often in *Enterobacter* species and *Klebsiella* species, but also occurs in *E. coli* and other genera. It most markedly affects ertapenem; isolates may remain susceptible to other carbapenems at breakpoint concentrations, but often show some degree of reduced susceptibility or resistance, with the level contingent upon the amount of ESBL/AmpC activity and the precise nature of the porin lesion(s) (see Figure 1).

Figure 1. The problem with spotting the carbapenemase producers



Courtesy of Professor Neil Woodford, Public Health England.

- In *P. aeruginosa*, by far the commonest mode of carbapenem resistance is loss of OprD porin, and isolates only resistant to imipenem, but not other β-lactams are certain to have this mechanism. Meropenem, though not imipenem, is also affected by upregulated efflux in *P. aeruginosa*[21]. Most *P. aeruginosa* isolates that are resistant to both imipenem and meropenem will have both mutational mechanisms (perhaps also with derepressed AmpC) rather than a carbapenemase.
- Non-carbapenemase mechanisms have been claimed in *Acinetobacter*, but may reflect failure to detect weak OXA carbapenemases, rather than their absence.

In the face of the diversity of enzyme types, the considerable variation in levels of phenotypic carbapenem resistance (for example, in MIC evaluations), and the added complexity of non-carbapenemase-mediated carbapenem resistance, there is no universally applicable method to detect readily all mechanisms of carbapenem resistance.

The ideal indicator carbapenem is one to which all carbapenemases confer resistance, even when production is insufficient. No single carbapenem satisfies this criterion for all host species (Enterobacterales and non-fermenters).

The strongest advice is for laboratory staff to have a high index of suspicion when screening clinical samples or preliminary detection of carbapenem resistance in cultured isolates for reduced carbapenem susceptibility or resistance (see Figure 1).

All suspected isolates must be followed up with confirmatory tests locally and if necessary submit to a referral laboratory following current EUCAST recommendations.

Identification to genus/species level is highly desirable for the interpretation of resistance patterns. Identify at least to genus level all isolates found resistant to any of the indicator carbapenems, to ensure that reduced susceptibility or resistance is not an intrinsic trait. Identify to species level if the genus is not known to produce intrinsic carbapenemases.

4.2.1 Detection of carbapenem resistance in screening samples (Setting:1)

Culture remains highly useful for the isolation of CPE from stool samples or rectal swabs either as a stand-alone method or as a complement to molecular methods. Molecular methods such as PCR can provide rapid results in as little as 1 hr, which can be a significant advantage, whereas culture typically requires at least 18 hr incubation. When a PCR method indicates the presence of a carbapenemase gene(s), subsequent culture is necessary to determine whether the gene is actually harboured by Enterobacterales rather than other species such as *Pseudomonas* spp., *Acinetobacter* spp. or other glucose non-fermenters[22,23]. Furthermore, isolation of CPE by culture enables antimicrobial susceptibility testing and, when necessary, epidemiological typing. When PCR is negative, culture may be useful to detect CPE with less common carbapenemase genes that may not be targeted by the PCR assay[24].

There is no 'gold standard' method for the isolation of CPE in stool samples or rectal swabs, but a wide range of different culture media has been proposed[25,26] . There are several commercially available chromogenic media designed for the isolation of CPE and/or carbapenem-resistant Enterobacterales (CRE) that are effective for isolation of CPE including those producing OXA-48-like enzymes. Chromogenic media for CPE incorporate antimicrobials for the inhibition of other microorganisms and two or more chromogenic substrates to differentiate key target species or groups of species as coloured colonies[27]. Their exact composition is often undisclosed and is subject to change over time.

It is not possible to provide firm recommendations to use a specific commercial chromogenic medium, however, a review of the published literature can help laboratory staff to make an informed choice. Readers are referred to Perry 2017, which includes a summary of detailed evaluations published to date[27].

Readers are advised to be cautious in the interpretation of study data. In all such studies, the calculation of sensitivity and specificity is based on the supposition that all isolates of CPE will be successfully detected by at least one of the methods under evaluation – although this may not actually be the case. The performance of a particular method may also be exaggerated if it is assessed alongside a relatively poor comparator. Finally, most studies are performed in a single location where a single type of carbapenemase is likely to predominate, and different types of media may show varied performances due to diverse geographical locations.

Despite these limitations, it is possible to draw some general conclusions. Producers of OXA-48-like enzymes are known to be difficult to detect because they frequently show low MICs to carbapenems. Certain media have shown limited sensitivity for

detection of such strains[25,28, 29, 30]. The problem with detection of strains with low carbapenem MICs has led some to advocate the use of chromogenic media designed for detection of ESBL producers [31]. This is not recommended for routine use owing to a lack of specificity in some studies[32], (due to the growth of ESBL-producers) and the inhibition of a proportion of strains with OXA-48-like enzymes that are susceptible to cephalosporins. Since these early studies, better options for specific detection of CPE are now available.

Media which have been evaluated specifically with KPC producers may have not been evaluated fully against certain other carbapenemase producers.

Broth media containing ertapenem or meropenem (at 2 mg/L) have been widely used following early practical recommendations made by the Centers for Disease Control and Prevention (CDC) in 2009 (and before the widespread availability of specific chromogenic media). A number of studies have since demonstrated an inferior performance in terms of both sensitivity and specificity when compared with chromogenic media for CPE[25, 31, 33,]. Furthermore, the CDC broth enrichment method requires an additional day to generate results. More promising results have been obtained by the use of other broth formulations followed by subculture onto chromogenic media. For example, an increased recovery of CPE with OXA-48-like enzymes has been demonstrated by use of an enrichment step using un-supplemented MacConkey broth[30] and nutrient broth plus 10 mg/L temocillin[29]. These findings are based on single reports and further studies are warranted.

Several reports have explored the use of non-selective media (e.g. MacConkey agar) in combination with carbapenem discs. Such methods can be compromised by overgrowth of carbapenem resistant non-carbapenemase producers and the risk of low inocula of CPE that have low carbapenem MICs appearing susceptible to a carbapenem disc. Several studies have shown equivalent performance to chromogenic agar but more recent studies have demonstrated reduced sensitivity. The use of ertapenem discs has greater sensitivity in comparison with other carbapenem discs, at a price of reduced specificity. These methods may be of value in small laboratories which would otherwise have to send samples to a referral laboratory [34,35].

It can be concluded that chromogenic media for CPE have advantages over the CDC broth method or the use of MacConkey-based agars supplemented with a carbapenem or used in conjunction with carbapenem discs. However, it is difficult to establish which, if any, chromogenic medium is optimal for detection of CPE in any particular location due to the different types of carbapenemase that may be encountered and the dominance of particular types in certain geographical regions.

There is very little evidence that extended incubation enhances the sensitivity of chromogenic media for CPE, but there is evidence to show that specificity is decreased[36].

There is only a small amount of evidence to support particular chromogenic media for the detection of carbapenemase-producing *Acinetobacter* species[27,37]. Similarly, there is no specific evidence for detecting carbapenemase-producing *Pseudomonas* species. Media containing ertapenem are not appropriate as *Acinetobacter* spp. are intrinsically resistant to this carbapenem.

This UK SMI recommends use of chromogenic agar for detection of carbapenemase producing Enterobacterales. It is recognised that some laboratories with low

throughput will not be able to maintain stocks of chromogenic media with all the necessary quality assurance. Furthermore, forwarding of samples to a referral laboratory entails a significant increase in turnaround time, which has infection control implications. In such circumstances, alternative methods may be used; however, this should be subject to local risk assessment. These include use of MacConkey or CLED with an ertapenem disc[33,38] using the screening cut-off of 27 mm[39]. Any suspect isolates must be subjected to full susceptibility testing in accordance with EUCAST recommendations.

It must be remembered that the EUCAST recommendation cut off of 25mm[40] applies to isolates that are recovered as a confluent growth. If the growth appears lighter than that stipulated for disc testing by EUCAST, colonies will require formal susceptibility testing.

4.2.2 Preliminary detection of carbapenem resistance from clinical samples (Setting 2)

This UK SMI supports the EUCAST recommendation to use meropenem as the indicator carbapenem as it offers the best compromise between sensitivity and specificity[41]. If inclusion of a meropenem disc in the routine first-line susceptibility panel is impractical, co-amoxiclav must be included in the testing panel on all such isolates. Any suspect isolates with co amoxiclav resistance or reduced susceptibility to meropenem must be subjected to full susceptibility testing in accordance with EUCAST recommendations. Although ertapenem has greater sensitivity, it is not recommended because it has poor specificity for carbapenemase producers (with the exception of screening on MacConkey/CLED). Faropenem has also been reported to show good sensitivity for detecting carbapenemase producers and the discs are commercially available[42].

4.2.3 Difficulties with detection of carbapenemase production in non-fermenters

Acquired carbapenemases are encountered in *Acinetobacter* species, *Pseudomonas* species (most commonly, though not exclusively in *P. aeruginosa)* and in other non-fermenters[5,6,17].

Consider testing meropenem, imipenem or doripenem against all clinically-significant isolates, as these have the right combination of sensitivity and specificity.

Do not use ertapenem because these species are intrinsically resistant to this carbapenem.

Carbapenem-resistant *Acinetobacter* species:

Isolates can usually be reported as likely OXA-carbapenemase producers without supplementary tests, unless the affected patient has been hospitalised overseas recently (for example, in the Middle-East or Indian subcontinent) in which case imipenem-EDTA or meropenem/dipicolinic acid (DPA) synergy[43,44] (>=8-fold) may be of value and could be sought to rule out the presence of a metallo-carbapenemase.

EDTA/DPA synergy testing in *Acinetobacter* species:

Strong EDTA or dipicolinic acid (DPA) synergy (>=8-fold) correlates well with MBL production in *Acinetobacter* species, although many OXA carbapenemase producers

show a weaker false EDTA synergy probably because metal ions are needed to maintain some OXA enzymes in an active conformation. A high false-positive rate in EDTA synergy tests may also arise due to growth inhibition of *Acinetobacter* species by EDTA alone.

Carbapenem-resistant *Pseudomonas* species:

Isolates resistant only to carbapenems can be inferred to have mutational resistance and need not be investigated further. However, isolates resistant to all relevant carbapenems (that is, imipenem, meropenem and doripenem), ceftazidime, ceftolozane/tazobactam and piperacillin/tazobactam may be tested for strong (>=8 fold) imipenem-EDTA or meropenem/DPA synergy[43,44]. Positives require further investigation using a molecular or an immunochromatographic assay. False-positive 'MBL' synergy is common and probably reflects the disorganising effects of EDTA on the outer membrane of some strains.

Aztreonam susceptibility in *Pseudomonas* species:

Susceptibility to aztreonam combined with resistance to carbapenems and other β-lactams is the 'classic' MBL phenotype, but many MBL producers are resistant to aztreonam owing to additional mechanisms meaning that the 'classic' pattern is not always seen. It should be noted that although carbapenem resistance is very common in isolates from cystic fibrosis patients, acquired carbapenemases are rare in this patient group[45].

Detection of KPC, OXA-48-like and GES-5 enzymes in non-fermenters:

GES-5, SIM, DIM-1, SPM-1 and the class D OXA-181 enzyme have also been reported in *P. aeruginosa* isolates (representing <10% of confirmed carbapenemase-producing *Pseudomonas* species referred to the AMRHAI Reference Unit, PHE unpublished data)[46,47]. At this time, it is not possible to recommend sensitive and specific phenotypic criteria to infer the presence of KPC, OXA-48-like and GES-5 non-metallo-carbapenemases in non-fermenters.

4.3 Difficulties around reporting carbapenem susceptibility for carbapenemase producing Enterobacterales

There is a division of opinion about the reporting of carbapenem susceptibility for carbapenemase producers. There has been expert opinion for several years that all carbapenemase producers should be reported resistant to all carbapenems, irrespective of susceptibility test results. However, this approach has been superseded by EUCAST recommendation of reporting susceptibility testing in accordance with breakpoints[40].

EUCAST have taken the view to adopt the low breakpoints, carbapenem susceptibility results can be taken at face value, and that carbapenems can be used as therapy so long as carbapenemase producers appear susceptible *in vitro*.

There is a need for more evidence of clinical success for carbapenems against carbapenemase producers with low MICs. Furthermore, 'susceptible' MIC and zone test results for carbapenemase producers often have poor reproducibility, with discrepant results between methods. There is a need to improve the quality of laboratory testing and reporting[48].

The best advice is to apply utmost caution if carbapenems are to be used in severe infections due to known carbapenemase producers, and to avoid using them as monotherapy[18].

New β-lactam and β-lactam/β-lactamase inhibitor combinations that have activity against some carbapenemases (principally KPC types, not MBLs) are under development or have been licensed by the European Medicines Directorate[49].

4.4 Other methods for detection of carbapenemases

Other methods that may also be considered for detecting likely carbapenemase producers include:

Carbapenem Inactivation Method (CIM): a phenotypic test developed to detect carbapenemase activity by incubating a carbapenem disc within the test bacterial suspension. Following two hours incubation the disc is placed on an agar plate inoculated with *E. coli* ATCC 25922 and incubated for a minimum of six hours. Inactivation of the carbapenem due to carbapenemase activity will produce no zone around the disc, whereas no carbapenemase activity will produce a zone[50]. Different variants of the CIM test have been published[51], which report improvements over the original version.

Biochemical tests: Some tests provide rapid (<2hr) detection of carbapenem hydrolysis. These tests are based on the classical acidometric penicillinase test whereby the pH change arising from carbapenem hydrolysis results in a red to yellow colour change with phenol red and a blue to green/yellow change with bromophenol blue[41]. These tests have been reported to work well for detecting carbapenemases in Enterobacterales and *Pseudomonas* species[52] but can be less reliable for *Acinetobacter* species[53] depending on the version used.

Modified Hodge Test (MHT) or 'Cloverleaf' test: is a phenotypic bioassay to assess the ability of a test strain to hydrolyse carbapenems. However, it is not recommended by EUCAST due to concerns over its specificity and sensitivity, with several proven carbapenemase producers giving consistently negative results[41].

Synergy tests are most effective for members of the Enterobacterales. Although EDTA/dipicolinic acid-based synergy tests may also be useful for non-fermenters, EDTA-based tests give a high proportion of false-positive results for these organisms. Check individual test instructions for use to ensure that the product can be used for non-fermenters.

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-ToF MS): MALDI-ToF MS offers the potential to detect carbapenemase production via detection of mass changes that follow hydrolysis of a carbapenem molecule[54-56]. It requires pre-incubation of a carbapenem with the test organism but can be completed in less than 2 hours and gives a 'Yes / No' result. Several 'in-house' versions have been published and the methodology has been commercialised as the MBT STAR-Carba assay[57].

Commercial assays: There are numerous commercial PCR-based and immunochromatographic assays available for detection of acquired carbapenemase genes or epitopes from bacterial cultures, screening swabs or clinical specimens. This UK SMI recommends implementation of an assay to detect at least the 'big 4' families (KPC, OXA-48-like, NDM and VIM). PHE has published a report to provide the

evidence base for diagnostic laboratories to facilitate an informed choice on the commercial assay to implement[58].

Automated or semi-automated systems generally can be used to detect carbapenem resistance though the ability of software to infer and warn correctly of the presence of carbapenemases is variable, especially for OXA-48-like enzymes[59]. For this reason, the underlying resistance mechanisms inferred by expert algorithms should be viewed with caution; some warn of potential carbapenemase production by every carbapenem-resistant isolate (good sensitivity and poor specificity) while others attempt to distinguish true carbapenemase producers from those with other mechanisms, which reduces their sensitivity. Studies on isolates with KPC carbapenemases indicate poor agreement between the MICs found by automated susceptibility systems[10,59,60]

4.5 Summary of UK SMI recommendations

Setting 1: Recommendations for screening of clinical samples

This UK SMI recommends the use of chromogenic agar for detection of carbapenemase producers. However, subject to local risk assessment laboratories with low throughput should consider using MacConkey or CLED with an ertapenem disc using zone size cut off of 27mm[39] (see section 4.2.1).

Setting 2: Recommendation for preliminary detection of carbapenem resistance in cultured isolates from clinical samples

This UK SMI recommends the routine use of meropenem as the indicator carbapenem. In situations where indicator carbapenem cannot be included in the primary susceptibility testing panel, any suspect isolates with co-amoxiclav resistance or reduced susceptibility to meropenem isolates must be subjected to full susceptibility testing in accordance with EUCAST recommendations (see section 4.2.2).

5. Safety considerations[61-77]

5.1 Specimen collection, transport and storage[61-66]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

5.2 Specimen processing[61-73,75-77]

Hazard Group 2 organisms.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[69].

Refer to current guidance on the safe handling of all organisms discussed in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

6. Investigation

6.1 Culture of carbapenemase producers

6.1.1 Specimen type

Any sample type can be used however rectal specimens (swabs with visible faecal material or discoloration) are the most sensitive for detecting CPE colonisation or a faecal specimen. If a rectal swab is not feasible or acceptable any clinical specimens such as blood, wound swab or urine is suitable.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage:

For safety considerations refer to Section 5.

Collect specimens before starting antimicrobial therapy where possible[78].

A single rectal swab is sufficient to determine CPE colonisation status on admission unless patients have previously been identified as CPE positive (in which case hospitals may wish to treat these patients as persistently colonised regardless of screening). In addition, if the patient has been hospitalised in a country with reported high prevalence of carbapenemase producers, include samples from any wounds or device-related sites[78].

In high risk situations, single swabs have poor negative predictive value. While recognising the limited sensitivity of a single swab the PHE's CPE framework recommends that a single swab is sufficient to determine the CPE colonisation status on admission in otherwise non-high risk situations.

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium[79-83].

Specimens should be transported and processed as soon as possible[78].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[78].

6.1.3 Laboratory processes (analytical stage)

Culture

Sample preparation

For safety considerations refer to Section 5.

Specimen processing

Stool samples and rectal swabs are used for screening for carbapenemases but generally rectal swabs are received.

Table 2: Investigation

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	**Atmos**	**Time**		
Setting 1: Screening of clinical samples[a]: Any condition + detection of carbapenem resistance/ carbapenemase production	Any sample	Chromogenic agar with carbapenem [b]/ Chromogenic medium for CPE **OR**	Refer to manufacturer's instructions [b]				Carbapenemase producing Enterobacterales
		If chromogenic agar not available, MacConkey / CLED agar + 10µg ETP disc [c] [39]	35-37	Aerobic	16-48hr	≥16hr [d]	Any carbapenemase producing Gram negative organism [e]
Setting 2: Preliminary detection of carbapenem resistance in cultured isolates from clinical samples: Routine Susceptibility testing of clinical samples	Any sample	Routine susceptibility testing media against co-amoxiclav [f] (minimum) or ideally meropenem.	35-37	Aerobic	18-24hr	≥18hr	Any carbapenemase producing Gram negative organisms

a Any suspect carbapenem resistant isolate should be followed up with susceptibility testing in accordance with EUCAST recommendations.
b For chromogenic media, refer to manufacturer's instructions for recommended incubation times.
c Ertapenem = ETP

d This UK SMI recommends zone size cut off of 27mm. Any suspect isolates should be submitted for full sensitivity testing in accordance with EUCAST recommendations.

e When investigating an outbreak of *Acinetobacter* species the incubation may have to be increased to 48 hours. Carriage of *Acinetobacter* using this method may be reduced.

f All co-amoxiclav resistant isolates should be screened for resistance or reduced susceptibility to carbapenems in accordance with EUCAST recommendations (if meropenem has not been tested).

Identification

Refer to UK SMI mentioned below for organism identification.

Minimum level of identification in the laboratory

All Enterobacterales	species level ID 16 - Identification of Enterobacterales
Pseudomonas species *Acinetobacter* species	species level ID 17 - Identification of *Pseudomonas* species and other non-glucose fermenters

Note: From 1 October 2020 diagnostic laboratories in England will have a duty to report acquired carbapenemase-producing Gram-negative bacteria identified in human samples to PHE. Further to this, diagnostic laboratories in England will have a duty to report the results of any antimicrobial susceptibility test and any carbapenem resistance mechanism identified in any of the causative agents listed in Schedule 2 of the Regulations[84], where this is known to the operator.

Technical limitations

Specimen containers: UK SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Quality control: The carbapenem discs that are used should be quality control tested, using disc diffusion methods and quality control strains, as described in accordance with EUCAST recommendations guideline[85]. Follow guidelines for frequency of disc quality control testing and corrective action if results are out of range.

Chromogenic media: Chromogenic media are affected by light and plates should be stored in the dark and not left in the light before or after inoculation. Inoculate culture media with a rectal swab or other sample (refer to Q 5 – Inoculation of culture media in bacteriology). Incubation times for chromogenic media should be as recommended by the manufacturers.

Microscopy

N/A

6.1.4 Post-laboratory processes (reporting procedures)

Culture

Interpreting and reporting results

Setting 1: Screening of clinical samples report culture result as:

Positive report

"Carbapenem-resistant / non-susceptible organism isolated. Further report on susceptibility mechanism to follow".

Negative report

"Carbapenem-resistant / non-susceptible organism not isolated"

Setting 2: Preliminary detection of carbapenem resistance in cultured isolates report as usual following confirmation of susceptibility testing.

Culture reporting time

Interim or preliminary results should be issued on detection of clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Microscopy

Interpreting and reporting results

N/A

Gram stain

N/A

Microscopy reporting time

N/A

7. Antimicrobial susceptibility testing

Use the media recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and refer to the respective guidelines.

Species identification is highly desirable to allow interpretation of results. Recommended clinical breakpoints for the carbapenems may be updated annually and should be sought from the links above.

Variations in enzyme expression and interplay with other host strain factors, mean that not all carbapenemase producers will show phenotypic resistance, that is, MICs for some or all carbapenems may lie below the clinical breakpoints or zone size diameters may be larger. Hence reliance on these values for detection of producers lacks sensitivity. EUCAST has therefore recommended screening cut-off values for detecting putative CPE[41].

7.1 Cultured isolates of Enterobacterales

Ideally all clinically significant isolates of Enterobacterales should be tested against a carbapenem. As a minimum testing for carbapenem resistance should be undertaken on all isolates from ‘high-risk’ patients and settings in accordance with current national guidance and any Enterobacterales isolates found resistant to co-amoxiclav or *Pseudomonas* isolates resistant to piperacillin-tazobactam.

Perform formal susceptibility testing in accordance to EUCAST recommendations. Refer to EUCAST guidelines for breakpoint information.

Laboratories should undertake further tests if automated systems flag any non-susceptibility to a carbapenem, irrespective of the expert interpretation given (unless it is explained by intrinsic resistance). Enterobacterales isolates resistant to the indicator carbapenem by clinical breakpoint or otherwise positive by the EUCAST screening criteria (see section 7) should be subjected to confirmatory tests[41].

Perform a molecular or immunochromatographic assay for at least the detection of KPC, OXA-48-like, NDM and VIM carbapenemase families on isolates.

If required, perform carbapenemase confirmatory tests as recommended by EUCAST on isolates found resistant or to have reduced susceptibility to the indicator carbapenem.

EUCAST advocate that supplemental tests to confirm carbapenemase production are unnecessary for individual patient management; the only test needed is MIC determination – either by agar or broth dilution, or by use of gradient strip methods[6,59]. The risk of onward spread may vary with underlying resistance mechanisms. EUCAST indicate the value of supplemental testing for infection prevention and control purposes, and for local epidemiological investigations.

7.2 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

7.2.1 Controls for carbapenemase tests

Quality control of the carbapenem discs used in the screening should follow standard EUCAST recommendations.

Positive controls should be used to ensure the performance of carbapenemase confirmatory tests. Various strains, including strains that express known carbapenemases and EUCAST recommended control strains are available from Public Health England's National Collection of Type Cultures (NCTC) (https://www.phe-culturecollections.org.uk/media.aspx?pid=182182). The NCTC online catalogue can be searched using the NCTC numbers listed below. Alternatively, some may be obtained commercially from other suppliers.

Table 3. Selected control strains producing carbapenemases available from the NCTC

Class A Carbapenemases		
Organism	**NCTC strain reference**	**Characteristics**
Klebsiella pneumoniae	NCTC 13438	Member of the international ST258 clone producing KPC-3 non-metallo-carbapenemase
	NCTC 14327	KPC-3 non-metallo-carbapenemase
	NCTC 14384	KPC-33 non-metallo-carbapenemase
Escherichia coli	NCTC 13919	GES-5 non-metallo-carbapenemase
	NCTC 14320	KPC non-metallo-carbapenemase IMP metallo-carbapenemase OXA-48-like non-metallo-carbapenemase

	NCTC 14321	KPC non-metallo-carbapenemase OXA-48-like non-metallo-carbapenemase
Serratia marcescens	NCTC 13920	SME-4 non-metallo-carbapenemase
Enterobacter cloacae / complex	NCTC 13922	NMC-A non-metallo-carbapenemase
	NCTC 13925	IMI-2 non-metallo-carbapenemase
	NCTC 14322	KPC-4 non-metallo-carbapenemase
	NCTC 14336	KPC-2 non-metallo-carbapenemase
Enterobacter asburiae	NCTC 14055	FRI-2 non-metallo-carbapenemase
Class B Carbapenemases (Metallo-β-lactamases)		
Organism	**NCTC strain reference**	**Characteristics**
Pseudomonas aeruginosa	NCTC 13437	VIM-10 metallo-carbapenemase; VEB-1 ESBL
	NCTC 13921	SPM-1 metallo-carbapenemase
	NCTC 14361	SIM metallo-carbapenemase
Pseudomonas guariconensis	NCTC 14056	DIM-1 metallo-carbapenemase
Klebsiella pneumoniae	NCTC 13439	VIM-1 metallo-carbapenemase
	NCTC 13440	VIM-1 metallo-carbapenemase
	NCTC 13443	NDM-1 metallo-carbapenemase
	NCTC 14323	NDM-1 metallo-carbapenemase; OXA-48 non-metallo-carbapenemase
	NCTC 14331	NDM-1 metallo-carbapenemase

	NCTC 14332	NDM-1 metallo-carbapenemase; OXA-232 non-metallo-carbapenemase
	NCTC 14334	IMP-4 metallo-carbapenemase
	NCTC 14337	IMP-1 metallo-carbapenemase
Escherichia coli	NCTC 13476	IMP-type metallo-carbapenemase
	NCTC 14320	IMP metallo-carbapenemase; KPC non-metallo-carbapenemase; OXA-48-like non-metallo-carbapenemase
	NCTC 14325	NDM-7 metallo-carbapenemase
	NCTC 14333	NDM-4 metallo-carbapenemase
	NCTC 14339	NDM-5 metallo-carbapenemase
Salmonella Seftenberg	NCTC 13953	NDM-1 metallo-carbapenemase
Enterobacter cloacae	NCTC 14326	VIM-1 metallo-carbapenemase
	NCTC 14328	VIM-4 metallo-carbapenemase
Citrobacter freundii	NCTC 14089	GIM-1 metallo-carbapenemase
Class D Carbapenemases (OXA carbapenemases)		
Organism	**NCTC strain reference**	**Characteristics**
Acinetobacter baumannii	NCTC 13301	OXA-23-like (and intrinsic OXA-51-like)
	NCTC 13302	OXA-25-like (OXA-24/40-like) (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13303	OXA-26 (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13304	OXA-27 (and intrinsic OXA-51-like) non-metallo-carbapenemases

	NCTC 13305	OXA-58-like (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13420	OXA-51-like non-metallo-carbapenemase
Klebsiella pneumoniae	NCTC 13442	Sequence type 353 with OXA-48-like
	NCTC 14323	OXA-48 non-metallo-carbapenemase; NDM-1 metallo-carbapenemase
	NCTC 14330	OXA-181 non-metallo-carbapenemase
	NCTC 14332	OXA-232 non-metallo-carbapenemase; NDM-1 metallo-carbapenemase
	NCTC 14335	OXA-232 non-metallo-carbapenemase
Escherichia coli	NCTC 14320	OXA-48-like non-metallo-carbapenemase; IMP metallo-carbapenemase; KPC non-metallo-carbapenemase9
	NCTC 14321	OXA-48-like non-metallo-carbapenemase; KPC non-metallo-carbapenemase
	NCTC 14324	OXA-484 non-metallo-carbapenemase
	NCTC 14329	OXA-244 non-metallo-carbapenemase
	NCTC 14338	OXA-48 non-metallo-carbapenemase
Salmonella Typhimurium	NCTC 13954	OXA-48 non-metallo-carbapenemase, as mediated by the pOXA-48a-like plasmid

Note: Either *Escherichia coli* NCTC 10418 or NCTC 12241 (equivalent to ATCC 25922) should be used as a negative control in confirmation tests.

A lenticule® disc including NCTC positive controls for the 'big 5' carbapenemases is available from NCTC (https://www.phe-culturecollections.org.uk/products/bacteria/antimicrobial-resistance-gene-controls.aspx). To assist with local validation of in-house or commercial molecular and

immunochromatographic assays a panel of CPE isolates (NCTC 14320 – NCTC 14339 consecutively, and listed above) representing common variants of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases known to be circulating in the UK can also be obtained from the NCTC.

8. Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see PHE user manuals and request forms.

Frontline diagnostic laboratories are strongly recommended to implement a PCR or immunochromatographic assay for detection of the 'big 4' carbapenemase families[58] in any isolate that appears to be resistant to the indicator carbapenem. Some PHE Specialist Laboratories offer referral services at a regional level, and then refer selected isolates onwards to the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit in Colindale. Laboratories using this regional service should not submit isolates to AMRHAI directly. All carbapenemase producers confirmed by diagnostic laboratories or PHE Specialist Laboratories from normally sterile sites only should be referred to AMRHAI for inclusion in the national strain archive. Refer to PHE's Bacteriology Reference Department user manual for up-to-date guidance on bacterial isolates that should be referred to the AMRHAI Reference Unit, turnaround times, transport procedure and the other requirements.

From 1 October 2020, diagnostic laboratories in England will have the duty to report the following to PHE:

- Acquired carbapenemase-producing Gram-negative bacteria isolated from human samples
- The results of any antimicrobial susceptibility test and any resistance mechanism identified in any of the causative agents listed in Schedule 2 of the Regulations.

Contact the appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit
Bacteriology Reference Department
National Infection Service
Public Health England
61 Colindale Avenue
London
NW9 5EQ

https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services

Telephone: +44 (0) 208 3277887

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Wales

Specialist Antimicrobial Chemotherapy Unit
Public Health Wales Microbiology Cardiff
University Hospital of Wales
Heath Park
Cardiff

CF14 4XW

Specialist Antimicrobial Chemotherapy Unit (SACU) 2021 - Public Health Wales (nhs.wales)

Scotland

https://www.hps.scot.nhs.uk/a-to-z-of-topics/reference-laboratories/#publications

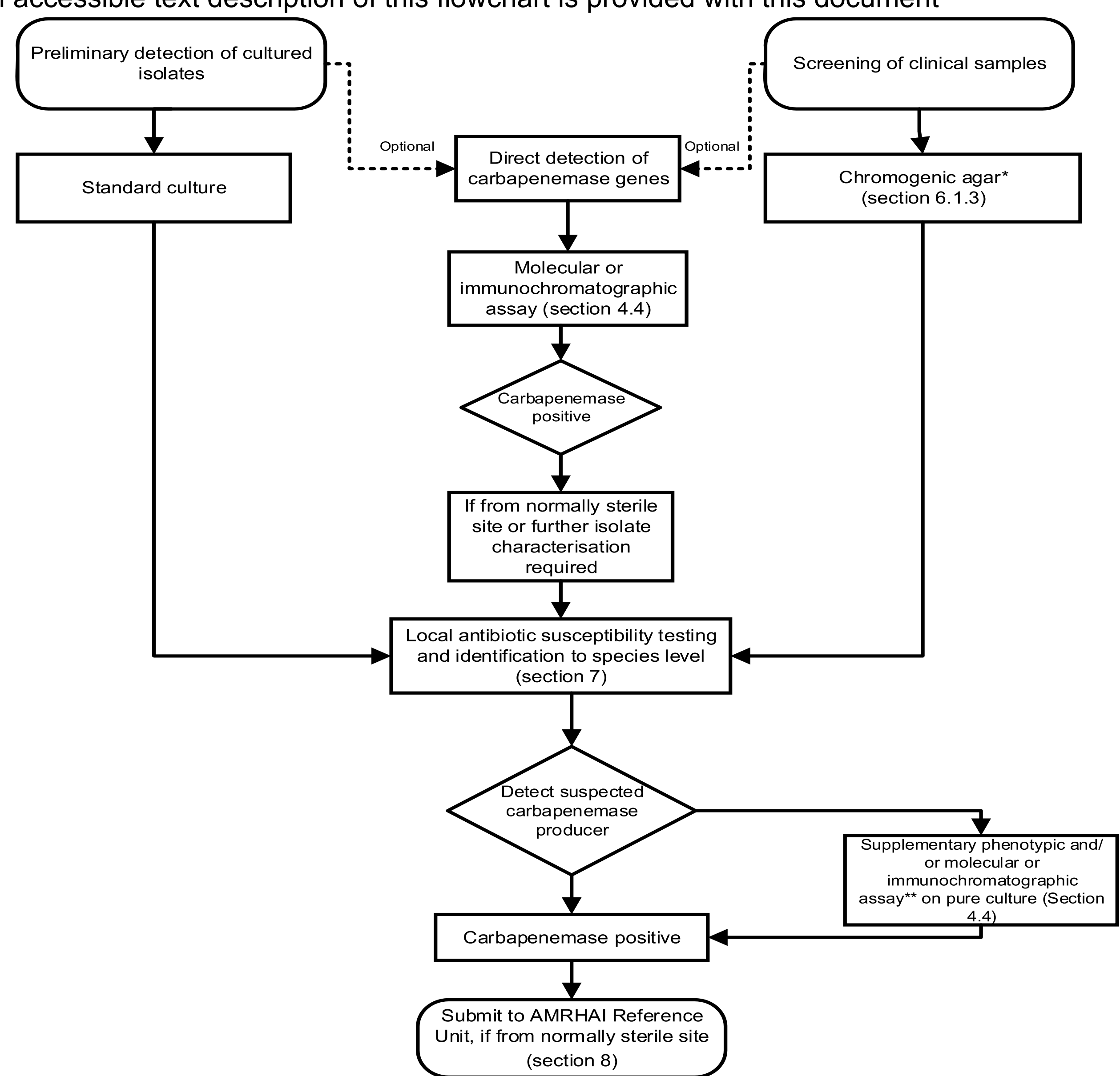
Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix 1: Flowchart for the detection of carbapenemases on cultured isolates and screening samples

An accessible text description of this flowchart is provided with this document



*If not available use MacConkey / CLED agar + 10µg ETP disc.
**As a minimum perform molecular or immunochromatographic detection of the ‘big 4’ (KPC, OXA-48-like, NDM and VIM). Isolates that are negative for the ‘big 4’ carbapenemase families should also be referred to the AMRHAI Reference Unit to rule out presence of rarer carbapenemase families.

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UK Standards for Microbiology Investigations

Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)

NICE accredited www.nice.org.uk/accreditation

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, National Infection Service, PHE

Bacteriology | B 60 | Issue no: 3.1 | Issue date: 01.06.2022 | Page: 1 of 32

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Contents

Acknowledgments **2**

Amendment table **4**

1. General information **6**

2. Scientific information **6**

3. Scope of document **6**

4. Background **6**

5. Safety considerations **15**

6. Investigation **16**

7. Antimicrobial susceptibility testing **19**

8. Referral to reference laboratories **24**

Appendix 1: Flowchart for the detection of carbapenemases on cultured isolates and screening samples **26**

References **27**

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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	5/01.06.2022
Issue number discarded	3
Insert issue number	3.1
Anticipated next review date*	18.09.2023
Section(s) involved	**Amendment**
Section 8: Referral to reference laboratories	Replaced 'Anaerobic reference unit (ARU) Cardiff' with 'Specialist Antimicrobial Chemotherapy Unit'.

Amendment number/date	4/18.09.2020
Issue number discarded	2.1
Insert issue number	3
Anticipated next review date*	18.09.2023
Section(s) involved	**Amendment**
Whole document	Document has been transferred to a new template. Clarity on different settings when testing for carbapenemase producers added to the document. Headings changed. Clarification of use of selective media with carbapenem disc if chromogenic agar is not available. Whole document restructured for clarity
Section 6. Investigation	Investigation table amended. Updated to include new regulations regarding reporting of acquired carbapenemase-producing Gram-negative bacteria identified in human samples to PHE. Interpreting and reporting results for culture amended.

Section 7.2.1 Controls for carbapenemase tests	Control strains producing carbapenemases available from the NCTC amended.
Appendix 1	Flowchart for the detection of carbapenemases on cultured isolates and screening samples amended.
Appendix	Appendix 2 removed. Readers are referred to detailed evaluations published to date.
References	References reviewed and updated.

*Reviews can be extended up to five years subject to resources available.

1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

The UK SMI gives recommendations on screening and detection of acquired 'carbapenemases' (carbapenem-hydrolysing β-lactamases). It should be used in conjunction with any local documents and PHE's framework of actions to contain carbapenemase-producing Enterobacterales.

This UK SMI should be used in conjunction with other relevant UK SMIs.

This document is intended to refer to different settings when testing for carbapenemase producers:

- Setting 1: screening of clinical samples for the presence of carbapenem resistance in particular carbapenemases
- Setting 2: preliminary detection of carbapenem resistance in cultured isolates from clinical samples.
- Formal confirmation of carbapenem resistance by susceptibility testing of suspected isolates.

We are following EUCAST recommendations for Setting 2 and formal confirmation of carbapenem resistance. There are no agreed international recommendations in place for Setting 1.

4. Background

The term 'carbapenemase' is used to mean any β-lactamase that hydrolyses carbapenems. Carbapenems (any or all of doripenem, ertapenem, imipenem and meropenem) are antimicrobials of last resort and are crucial for preventing and treating life-threatening nosocomial infections. Carbapenemases are clinically important because they destroy, and so may confer resistance to, carbapenems (and usually most other β-lactams). Carbapenemases are found naturally in a few clinically relevant bacteria, such as *Stenotrophomonas maltophilia*, *Aeromonas* species, and 'chryseobacteria', including *Elizabethkingia meningoseptica*[1]. *Acinetobacter baumannii* also has the gene for an intrinsic carbapenemase (OXA-51-like), but this confers reduced susceptibility or resistance to carbapenems only when its expression is up-regulated by genetic reorganisation[2].

In addition, non-susceptibility or resistance to specific carbapenems is an intrinsic characteristic of some Gram-negative bacteria: most non-fermenters are naturally resistant to ertapenem (but not to other carbapenems); *Serratia* species and Proteeae have intrinsic poor susceptibility or low-level resistance to imipenem (but not to other carbapenems).

Studies have shown that *Bacteroides fragilis* can harbour a chromosomally mediated metallo-β-lactamase gene called cfiA (ccrA) which confers resistance to carbapenem[3,4].

This document focuses on acquired carbapenemases. Accurate identification of bacteria to genus or species level will allow laboratories to recognise the producers of intrinsic carbapenemases detailed above.

4.1 Acquired carbapenemases

Acquired carbapenemases are diverse and include three of four Ambler's molecular classes of β-lactamases[5,6].They are detailed below:

Class A enzymes: All hydrolyse carbapenems effectively and are partially inhibited by clavulanic acid. The most widespread carbapenemases in this class are the KPC enzymes; other, less frequently-encountered class A carbapenemases include some GES types (notably GES-5), IMI/NMC-A (in *Enterobacter* species), FRI (in *Enterobacter* species) and SME (in *Serratia marcescens*)[7]. KPC enzymes have been recorded in *A. baumannii* in Central America, and in *P. aeruginosa* in Central and South America, USA, China, the Caribbean, and occasionally in the UK[8-12 13,14].

Class B enzymes: Also known as 'metallo-β-lactamases' (MBLs) or 'metallo-carbapenemases'[5,6]. These differ fundamentally from all other β-lactamases because they require zinc ions in their active sites for activity[15]. Consequently, they are inactivated by metal ion chelators, such as EDTA[16]. The major MBL families encountered in the UK are the NDM, VIM and, less commonly, IMP types. Other types include GIM, SIM, DIM and SPM-1 enzymes, which have been found at a very low frequency in *P. aeruginosa* and *Citrobacter freundii* within the UK.

Class D enzymes: This class comprises many (>400) diverse β-lactamases, a few of which are carbapenemases[5,6,17]. Important carbapenemases within the family include OXA-23, -40, -51, -58 and their variants from *Acinetobacter* species and OXA-48-like enzymes in Enterobacterales; other rarer carbapenem-hydrolysing class D types include OXA-198 in *Pseudomonas* species.

Although some are chromosomally encoded (e.g. NMC-A/IMI and SME), many acquired carbapenemases are plasmid-mediated (especially when found in Enterobacterales), giving potential for spread between strains, species and genera.

Table 1. Carbapenemases currently known to be circulating in the UK by classification, activity and organisms

Enzyme type	Classification by ambler class	Activity spectrum	Organism(s)
KPC	A	All β-lactams	Enterobacterales; rare in *P. aeruginosa*
SME	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*S. marcescens*
NMC–A IMI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*Enterobacter* species; rare in other Enterobacterales
GES	A	Depends on enzyme variant. Some are ESBLs, others e.g. GES-5 are carbapenemases	*P. aeruginosa* and Enterobacterales
FRI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*Enterobacter* species

IMP **VIM** **NDM** GIM, DIM, SIM, SPM-1 (infrequently identified in the UK)	B (metallo-β-lactamases)	All β-lactams except monobactams (aztreonam)	*Pseudomonas* species; *Acinetobacter* species; Enterobacterales
OXA	D	Carbapenems (note that many OXA enzymes are NOT carbapenemases)	*A. baumannii*; Enterobacterales and rare in *P. aeruginosa*

Note: The enzyme types in bold are the five main carbapenemase families found in the UK, the so-called 'big five'.

Delayed recognition and inappropriate treatment of severe infections caused by carbapenemase producers is associated with increased mortality[18]. Many producers are multi-resistant to non-β-lactam antibiotics including quinolones and aminoglycosides.

The carbapenem MIC ranges for Enterobacterales producing each of the 'big five' carbapenemases (KPC, OXA-48-like, NDM, VIM and IMP) span from below the susceptible clinical breakpoints to high-level resistance, and when combined with the diversity of carbapenemase types, this means that few, if any, strategies reliably detect all carbapenemase producers. Nevertheless, the carbapenem MICs of most carbapenemase-producing bacteria will be above the epidemiological cut-off (ECOFF) values defined by EUCAST even if some isolates are not clinically resistant (that is, MICs remain equal to or below the clinical breakpoints). ECOFF values (or breakpoints) mark the limit of the wild-type population by a statistical definition, and isolates with higher MICs/lower zone diameters represent non-wild-type isolates.

The level of carbapenem resistance displayed by some carbapenemase producers is a cause for concern. Higher MICs are observed when carbapenemase producers also lack major porins. Among strains with lower MICs and without porin loss there is potential for carbapenemase producers to spread undetected. This concern is greatest with OXA-48-like enzymes in Enterobacterales, which can give very low level carbapenem resistance[19], without cross-resistance to cephalosporins. KPC enzymes and MBLs tend to confer broader effects on the β-lactam resistance profile of the host strain.

4.2 Complexities of detection of carbapenemase production

Clinical laboratories should have a high index of suspicion while investigating isolates for carbapenemase production and should be alert to two confounders:

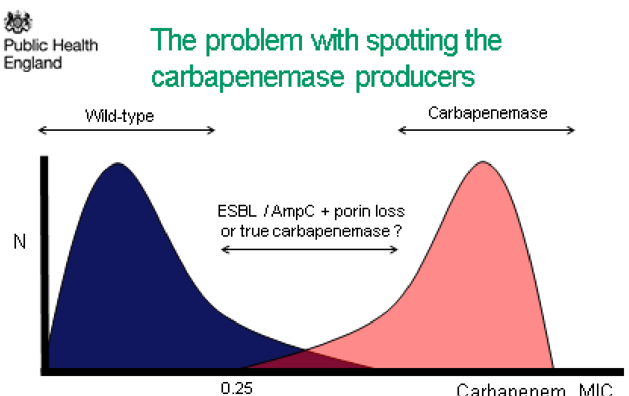
a) not all carbapenem-resistant isolates produce a carbapenemase (resistance can be mediated by other mechanisms, such as the combination of ESBL/AmpC plus impermeability, as below),

b) not all carbapenemase producers are resistant to carbapenems

Carbapenemases are not the only mechanism of acquired resistance to carbapenems but are the most important from a public health perspective. Other mechanisms include the following:

- Enterobacterales with ESBL or AmpC enzymes may lose outer membrane porins (through mutations or other disruptions in chromosomal genes), reducing carbapenem uptake[20]. In contrast to carbapenemases, these combinatorial mechanisms of carbapenem resistance are not transferable between strains (though the contributing ESBL might be) and the porin-deficient mutants may have reduced fitness and be less likely to spread in healthcare settings. This mechanism is seen most often in *Enterobacter* species and *Klebsiella* species, but also occurs in *E. coli* and other genera. It most markedly affects ertapenem; isolates may remain susceptible to other carbapenems at breakpoint concentrations, but often show some degree of reduced susceptibility or resistance, with the level contingent upon the amount of ESBL/AmpC activity and the precise nature of the porin lesion(s) (see Figure 1).

Figure 1. The problem with spotting the carbapenemase producers



Courtesy of Professor Neil Woodford, Public Health England.

- In *P. aeruginosa*, by far the commonest mode of carbapenem resistance is loss of OprD porin, and isolates only resistant to imipenem, but not other β-lactams are certain to have this mechanism. Meropenem, though not imipenem, is also affected by upregulated efflux in *P. aeruginosa*[21]. Most *P. aeruginosa* isolates that are resistant to both imipenem and meropenem will have both mutational mechanisms (perhaps also with derepressed AmpC) rather than a carbapenemase.
- Non-carbapenemase mechanisms have been claimed in *Acinetobacter*, but may reflect failure to detect weak OXA carbapenemases, rather than their absence.

In the face of the diversity of enzyme types, the considerable variation in levels of phenotypic carbapenem resistance (for example, in MIC evaluations), and the added complexity of non-carbapenemase-mediated carbapenem resistance, there is no universally applicable method to detect readily all mechanisms of carbapenem resistance.

The ideal indicator carbapenem is one to which all carbapenemases confer resistance, even when production is insufficient. No single carbapenem satisfies this criterion for all host species (Enterobacterales and non-fermenters).

The strongest advice is for laboratory staff to have a high index of suspicion when screening clinical samples or preliminary detection of carbapenem resistance in cultured isolates for reduced carbapenem susceptibility or resistance (see Figure 1).

All suspected isolates must be followed up with confirmatory tests locally and if necessary submit to a referral laboratory following current EUCAST recommendations.

Identification to genus/species level is highly desirable for the interpretation of resistance patterns. Identify at least to genus level all isolates found resistant to any of the indicator carbapenems, to ensure that reduced susceptibility or resistance is not an intrinsic trait. Identify to species level if the genus is not known to produce intrinsic carbapenemases.

4.2.1 Detection of carbapenem resistance in screening samples (Setting:1)

Culture remains highly useful for the isolation of CPE from stool samples or rectal swabs either as a stand-alone method or as a complement to molecular methods. Molecular methods such as PCR can provide rapid results in as little as 1 hr, which can be a significant advantage, whereas culture typically requires at least 18 hr incubation. When a PCR method indicates the presence of a carbapenemase gene(s), subsequent culture is necessary to determine whether the gene is actually harboured by Enterobacterales rather than other species such as *Pseudomonas* spp., *Acinetobacter* spp. or other glucose non-fermenters[22,23]. Furthermore, isolation of CPE by culture enables antimicrobial susceptibility testing and, when necessary, epidemiological typing. When PCR is negative, culture may be useful to detect CPE with less common carbapenemase genes that may not be targeted by the PCR assay[24].

There is no 'gold standard' method for the isolation of CPE in stool samples or rectal swabs, but a wide range of different culture media has been proposed[25,26] . There are several commercially available chromogenic media designed for the isolation of CPE and/or carbapenem-resistant Enterobacterales (CRE) that are effective for isolation of CPE including those producing OXA-48-like enzymes. Chromogenic media for CPE incorporate antimicrobials for the inhibition of other microorganisms and two or more chromogenic substrates to differentiate key target species or groups of species as coloured colonies[27]. Their exact composition is often undisclosed and is subject to change over time.

It is not possible to provide firm recommendations to use a specific commercial chromogenic medium, however, a review of the published literature can help laboratory staff to make an informed choice. Readers are referred to Perry 2017, which includes a summary of detailed evaluations published to date[27].

Readers are advised to be cautious in the interpretation of study data. In all such studies, the calculation of sensitivity and specificity is based on the supposition that all isolates of CPE will be successfully detected by at least one of the methods under evaluation – although this may not actually be the case. The performance of a particular method may also be exaggerated if it is assessed alongside a relatively poor comparator. Finally, most studies are performed in a single location where a single type of carbapenemase is likely to predominate, and different types of media may show varied performances due to diverse geographical locations.

Despite these limitations, it is possible to draw some general conclusions. Producers of OXA-48-like enzymes are known to be difficult to detect because they frequently show low MICs to carbapenems. Certain media have shown limited sensitivity for

detection of such strains[25,28, 29, 30]. The problem with detection of strains with low carbapenem MICs has led some to advocate the use of chromogenic media designed for detection of ESBL producers [31]. This is not recommended for routine use owing to a lack of specificity in some studies[32], (due to the growth of ESBL-producers) and the inhibition of a proportion of strains with OXA-48-like enzymes that are susceptible to cephalosporins. Since these early studies, better options for specific detection of CPE are now available.

Media which have been evaluated specifically with KPC producers may have not been evaluated fully against certain other carbapenemase producers.

Broth media containing ertapenem or meropenem (at 2 mg/L) have been widely used following early practical recommendations made by the Centers for Disease Control and Prevention (CDC) in 2009 (and before the widespread availability of specific chromogenic media). A number of studies have since demonstrated an inferior performance in terms of both sensitivity and specificity when compared with chromogenic media for CPE[25, 31, 33,]. Furthermore, the CDC broth enrichment method requires an additional day to generate results. More promising results have been obtained by the use of other broth formulations followed by subculture onto chromogenic media. For example, an increased recovery of CPE with OXA-48-like enzymes has been demonstrated by use of an enrichment step using un-supplemented MacConkey broth[30] and nutrient broth plus 10 mg/L temocillin[29]. These findings are based on single reports and further studies are warranted.

Several reports have explored the use of non-selective media (e.g. MacConkey agar) in combination with carbapenem discs. Such methods can be compromised by overgrowth of carbapenem resistant non-carbapenemase producers and the risk of low inocula of CPE that have low carbapenem MICs appearing susceptible to a carbapenem disc. Several studies have shown equivalent performance to chromogenic agar but more recent studies have demonstrated reduced sensitivity. The use of ertapenem discs has greater sensitivity in comparison with other carbapenem discs, at a price of reduced specificity. These methods may be of value in small laboratories which would otherwise have to send samples to a referral laboratory [34,35].

It can be concluded that chromogenic media for CPE have advantages over the CDC broth method or the use of MacConkey-based agars supplemented with a carbapenem or used in conjunction with carbapenem discs. However, it is difficult to establish which, if any, chromogenic medium is optimal for detection of CPE in any particular location due to the different types of carbapenemase that may be encountered and the dominance of particular types in certain geographical regions.

There is very little evidence that extended incubation enhances the sensitivity of chromogenic media for CPE, but there is evidence to show that specificity is decreased[36].

There is only a small amount of evidence to support particular chromogenic media for the detection of carbapenemase-producing *Acinetobacter* species[27,37]. Similarly, there is no specific evidence for detecting carbapenemase-producing *Pseudomonas* species. Media containing ertapenem are not appropriate as *Acinetobacter* spp. are intrinsically resistant to this carbapenem.

This UK SMI recommends use of chromogenic agar for detection of carbapenemase producing Enterobacterales. It is recognised that some laboratories with low

throughput will not be able to maintain stocks of chromogenic media with all the necessary quality assurance. Furthermore, forwarding of samples to a referral laboratory entails a significant increase in turnaround time, which has infection control implications. In such circumstances, alternative methods may be used; however, this should be subject to local risk assessment. These include use of MacConkey or CLED with an ertapenem disc[33,38] using the screening cut-off of 27 mm[39]. Any suspect isolates must be subjected to full susceptibility testing in accordance with EUCAST recommendations.

It must be remembered that the EUCAST recommendation cut off of 25mm[40] applies to isolates that are recovered as a confluent growth. If the growth appears lighter than that stipulated for disc testing by EUCAST, colonies will require formal susceptibility testing.

4.2.2 Preliminary detection of carbapenem resistance from clinical samples (Setting 2)

This UK SMI supports the EUCAST recommendation to use meropenem as the indicator carbapenem as it offers the best compromise between sensitivity and specificity[41]. If inclusion of a meropenem disc in the routine first-line susceptibility panel is impractical, co-amoxiclav must be included in the testing panel on all such isolates. Any suspect isolates with co amoxiclav resistance or reduced susceptibility to meropenem must be subjected to full susceptibility testing in accordance with EUCAST recommendations. Although ertapenem has greater sensitivity, it is not recommended because it has poor specificity for carbapenemase producers (with the exception of screening on MacConkey/CLED). Faropenem has also been reported to show good sensitivity for detecting carbapenemase producers and the discs are commercially available[42].

4.2.3 Difficulties with detection of carbapenemase production in non-fermenters

Acquired carbapenemases are encountered in *Acinetobacter* species, *Pseudomonas* species (most commonly, though not exclusively in *P. aeruginosa)* and in other non-fermenters[5,6,17].

Consider testing meropenem, imipenem or doripenem against all clinically-significant isolates, as these have the right combination of sensitivity and specificity.

Do not use ertapenem because these species are intrinsically resistant to this carbapenem.

Carbapenem-resistant *Acinetobacter* species:

Isolates can usually be reported as likely OXA-carbapenemase producers without supplementary tests, unless the affected patient has been hospitalised overseas recently (for example, in the Middle-East or Indian subcontinent) in which case imipenem-EDTA or meropenem/dipicolinic acid (DPA) synergy[43,44] (>=8-fold) may be of value and could be sought to rule out the presence of a metallo-carbapenemase.

EDTA/DPA synergy testing in *Acinetobacter* species:

Strong EDTA or dipicolinic acid (DPA) synergy (>=8-fold) correlates well with MBL production in *Acinetobacter* species, although many OXA carbapenemase producers

show a weaker false EDTA synergy probably because metal ions are needed to maintain some OXA enzymes in an active conformation. A high false-positive rate in EDTA synergy tests may also arise due to growth inhibition of *Acinetobacter* species by EDTA alone.

Carbapenem-resistant *Pseudomonas* species:

Isolates resistant only to carbapenems can be inferred to have mutational resistance and need not be investigated further. However, isolates resistant to all relevant carbapenems (that is, imipenem, meropenem and doripenem), ceftazidime, ceftolozane/tazobactam and piperacillin/tazobactam may be tested for strong (>=8 fold) imipenem-EDTA or meropenem/DPA synergy[43,44]. Positives require further investigation using a molecular or an immunochromatographic assay. False-positive 'MBL' synergy is common and probably reflects the disorganising effects of EDTA on the outer membrane of some strains.

Aztreonam susceptibility in *Pseudomonas* species:

Susceptibility to aztreonam combined with resistance to carbapenems and other β-lactams is the 'classic' MBL phenotype, but many MBL producers are resistant to aztreonam owing to additional mechanisms meaning that the 'classic' pattern is not always seen. It should be noted that although carbapenem resistance is very common in isolates from cystic fibrosis patients, acquired carbapenemases are rare in this patient group[45].

Detection of KPC, OXA-48-like and GES-5 enzymes in non-fermenters:

GES-5, SIM, DIM-1, SPM-1 and the class D OXA-181 enzyme have also been reported in *P. aeruginosa* isolates (representing <10% of confirmed carbapenemase-producing *Pseudomonas* species referred to the AMRHAI Reference Unit, PHE unpublished data)[46,47]. At this time, it is not possible to recommend sensitive and specific phenotypic criteria to infer the presence of KPC, OXA-48-like and GES-5 non-metallo-carbapenemases in non-fermenters.

4.3 Difficulties around reporting carbapenem susceptibility for carbapenemase producing Enterobacterales

There is a division of opinion about the reporting of carbapenem susceptibility for carbapenemase producers. There has been expert opinion for several years that all carbapenemase producers should be reported resistant to all carbapenems, irrespective of susceptibility test results. However, this approach has been superseded by EUCAST recommendation of reporting susceptibility testing in accordance with breakpoints[40].

EUCAST have taken the view to adopt the low breakpoints, carbapenem susceptibility results can be taken at face value, and that carbapenems can be used as therapy so long as carbapenemase producers appear susceptible *in vitro*.

There is a need for more evidence of clinical success for carbapenems against carbapenemase producers with low MICs. Furthermore, 'susceptible' MIC and zone test results for carbapenemase producers often have poor reproducibility, with discrepant results between methods. There is a need to improve the quality of laboratory testing and reporting[48].

The best advice is to apply utmost caution if carbapenems are to be used in severe infections due to known carbapenemase producers, and to avoid using them as monotherapy[18].

New β-lactam and β-lactam/β-lactamase inhibitor combinations that have activity against some carbapenemases (principally KPC types, not MBLs) are under development or have been licensed by the European Medicines Directorate[49].

4.4 Other methods for detection of carbapenemases

Other methods that may also be considered for detecting likely carbapenemase producers include:

Carbapenem Inactivation Method (CIM): a phenotypic test developed to detect carbapenemase activity by incubating a carbapenem disc within the test bacterial suspension. Following two hours incubation the disc is placed on an agar plate inoculated with *E. coli* ATCC 25922 and incubated for a minimum of six hours. Inactivation of the carbapenem due to carbapenemase activity will produce no zone around the disc, whereas no carbapenemase activity will produce a zone[50]. Different variants of the CIM test have been published[51], which report improvements over the original version.

Biochemical tests: Some tests provide rapid (<2hr) detection of carbapenem hydrolysis. These tests are based on the classical acidometric penicillinase test whereby the pH change arising from carbapenem hydrolysis results in a red to yellow colour change with phenol red and a blue to green/yellow change with bromophenol blue[41]. These tests have been reported to work well for detecting carbapenemases in Enterobacterales and *Pseudomonas* species[52] but can be less reliable for *Acinetobacter* species[53] depending on the version used.

Modified Hodge Test (MHT) or 'Cloverleaf' test: is a phenotypic bioassay to assess the ability of a test strain to hydrolyse carbapenems. However, it is not recommended by EUCAST due to concerns over its specificity and sensitivity, with several proven carbapenemase producers giving consistently negative results[41].

Synergy tests are most effective for members of the Enterobacterales. Although EDTA/dipicolinic acid-based synergy tests may also be useful for non-fermenters, EDTA-based tests give a high proportion of false-positive results for these organisms. Check individual test instructions for use to ensure that the product can be used for non-fermenters.

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-ToF MS): MALDI-ToF MS offers the potential to detect carbapenemase production via detection of mass changes that follow hydrolysis of a carbapenem molecule[54-56]. It requires pre-incubation of a carbapenem with the test organism but can be completed in less than 2 hours and gives a 'Yes / No' result. Several 'in-house' versions have been published and the methodology has been commercialised as the MBT STAR-Carba assay[57].

Commercial assays: There are numerous commercial PCR-based and immunochromatographic assays available for detection of acquired carbapenemase genes or epitopes from bacterial cultures, screening swabs or clinical specimens. This UK SMI recommends implementation of an assay to detect at least the 'big 4' families (KPC, OXA-48-like, NDM and VIM). PHE has published a report to provide the

evidence base for diagnostic laboratories to facilitate an informed choice on the commercial assay to implement[58].

Automated or semi-automated systems generally can be used to detect carbapenem resistance though the ability of software to infer and warn correctly of the presence of carbapenemases is variable, especially for OXA-48-like enzymes[59]. For this reason, the underlying resistance mechanisms inferred by expert algorithms should be viewed with caution; some warn of potential carbapenemase production by every carbapenem-resistant isolate (good sensitivity and poor specificity) while others attempt to distinguish true carbapenemase producers from those with other mechanisms, which reduces their sensitivity. Studies on isolates with KPC carbapenemases indicate poor agreement between the MICs found by automated susceptibility systems[10,59,60]

4.5 Summary of UK SMI recommendations

Setting 1: Recommendations for screening of clinical samples

This UK SMI recommends the use of chromogenic agar for detection of carbapenemase producers. However, subject to local risk assessment laboratories with low throughput should consider using MacConkey or CLED with an ertapenem disc using zone size cut off of 27mm[39] (see section 4.2.1).

Setting 2: Recommendation for preliminary detection of carbapenem resistance in cultured isolates from clinical samples

This UK SMI recommends the routine use of meropenem as the indicator carbapenem. In situations where indicator carbapenem cannot be included in the primary susceptibility testing panel, any suspect isolates with co-amoxiclav resistance or reduced susceptibility to meropenem isolates must be subjected to full susceptibility testing in accordance with EUCAST recommendations (see section 4.2.2).

5. Safety considerations[61-77]

5.1 Specimen collection, transport and storage[61-66]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

5.2 Specimen processing[61-73,75-77]

Hazard Group 2 organisms.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[69].

Refer to current guidance on the safe handling of all organisms discussed in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

6. Investigation

6.1 Culture of carbapenemase producers

6.1.1 Specimen type

Any sample type can be used however rectal specimens (swabs with visible faecal material or discoloration) are the most sensitive for detecting CPE colonisation or a faecal specimen. If a rectal swab is not feasible or acceptable any clinical specimens such as blood, wound swab or urine is suitable.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage:

For safety considerations refer to Section 5.

Collect specimens before starting antimicrobial therapy where possible[78].

A single rectal swab is sufficient to determine CPE colonisation status on admission unless patients have previously been identified as CPE positive (in which case hospitals may wish to treat these patients as persistently colonised regardless of screening). In addition, if the patient has been hospitalised in a country with reported high prevalence of carbapenemase producers, include samples from any wounds or device-related sites[78].

In high risk situations, single swabs have poor negative predictive value. While recognising the limited sensitivity of a single swab the PHE's CPE framework recommends that a single swab is sufficient to determine the CPE colonisation status on admission in otherwise non-high risk situations.

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium[79-83].

Specimens should be transported and processed as soon as possible[78].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[78].

6.1.3 Laboratory processes (analytical stage)

Culture

Sample preparation

For safety considerations refer to Section 5.

Specimen processing

Stool samples and rectal swabs are used for screening for carbapenemases but generally rectal swabs are received.

Table 2: Investigation

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	**Atmos**	**Time**		
Setting 1: Screening of clinical samples[a]: Any condition + detection of carbapenem resistance/ carbapenemase production	Any sample	Chromogenic agar with carbapenem [b]/ Chromogenic medium for CPE	Refer to manufacturer's instructions [b]				Carbapenemase producing Enterobacterales
		OR If chromogenic agar not available, MacConkey / CLED agar + 10µg ETP disc [c] [39]	35-37	Aerobic	16-48hr	≥16hr [d]	Any carbapenemase producing Gram negative organism [e]
Setting 2: Preliminary detection of carbapenem resistance in cultured isolates from clinical samples: Routine Susceptibility testing of clinical samples	Any sample	Routine susceptibility testing media against co-amoxiclav [f] (minimum) or ideally meropenem.	35-37	Aerobic	18-24hr	≥18hr	Any carbapenemase producing Gram negative organisms

a Any suspect carbapenem resistant isolate should be followed up with susceptibility testing in accordance with EUCAST recommendations.
b For chromogenic media, refer to manufacturer's instructions for recommended incubation times.
c Ertapenem = ETP
d This UK SMI recommends zone size cut off of 27mm. Any suspect isolates should be submitted for full sensitivity testing in accordance with EUCAST recommendations.

e When investigating an outbreak of *Acinetobacter* species the incubation may have to be increased to 48 hours. Carriage of *Acinetobacter* using this method may be reduced.

f All co-amoxiclav resistant isolates should be screened for resistance or reduced susceptibility to carbapenems in accordance with EUCAST recommendations (if meropenem has not been tested).

Identification

Refer to UK SMI mentioned below for organism identification.

Minimum level of identification in the laboratory

All Enterobacterales	species level ID 16 - Identification of Enterobacterales
Pseudomonas species *Acinetobacter* species	species level ID 17 - Identification of *Pseudomonas* species and other non-glucose fermenters

Note: From 1 October 2020 diagnostic laboratories in England will have a duty to report acquired carbapenemase-producing Gram-negative bacteria identified in human samples to PHE. Further to this, diagnostic laboratories in England will have a duty to report the results of any antimicrobial susceptibility test and any carbapenem resistance mechanism identified in any of the causative agents listed in Schedule 2 of the Regulations[84], where this is known to the operator.

Technical limitations

Specimen containers: UK SMIs use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

Quality control: The carbapenem discs that are used should be quality control tested, using disc diffusion methods and quality control strains, as described in accordance with EUCAST recommendations guideline[85]. Follow guidelines for frequency of disc quality control testing and corrective action if results are out of range.

Chromogenic media: Chromogenic media are affected by light and plates should be stored in the dark and not left in the light before or after inoculation. Inoculate culture media with a rectal swab or other sample (refer to Q 5 – Inoculation of culture media in bacteriology). Incubation times for chromogenic media should be as recommended by the manufacturers.

Microscopy

N/A

6.1.4 Post-laboratory processes (reporting procedures)

Culture

Interpreting and reporting results

Setting 1: Screening of clinical samples report culture result as:

Positive report

"Carbapenem-resistant / non-susceptible organism isolated. Further report on susceptibility mechanism to follow".

Negative report

"Carbapenem-resistant / non-susceptible organism not isolated"

Setting 2: Preliminary detection of carbapenem resistance in cultured isolates report as usual following confirmation of susceptibility testing.

Culture reporting time

Interim or preliminary results should be issued on detection of clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Microscopy

Interpreting and reporting results

N/A

Gram stain

N/A

Microscopy reporting time

N/A

7. Antimicrobial susceptibility testing

Use the media recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and refer to the respective guidelines.

Species identification is highly desirable to allow interpretation of results. Recommended clinical breakpoints for the carbapenems may be updated annually and should be sought from the links above.

Variations in enzyme expression and interplay with other host strain factors, mean that not all carbapenemase producers will show phenotypic resistance, that is, MICs for some or all carbapenems may lie below the clinical breakpoints or zone size diameters may be larger. Hence reliance on these values for detection of producers lacks sensitivity. EUCAST has therefore recommended screening cut-off values for detecting putative CPE[41].

7.1 Cultured isolates of Enterobacterales

Ideally all clinically significant isolates of Enterobacterales should be tested against a carbapenem. As a minimum testing for carbapenem resistance should be undertaken on all isolates from 'high-risk' patients and settings in accordance with current national guidance and any Enterobacterales isolates found resistant to co-amoxiclav or *Pseudomonas* isolates resistant to piperacillin-tazobactam.

Perform formal susceptibility testing in accordance to EUCAST recommendations. Refer to EUCAST guidelines for breakpoint information.

Laboratories should undertake further tests if automated systems flag any non-susceptibility to a carbapenem, irrespective of the expert interpretation given (unless it is explained by intrinsic resistance). Enterobacterales isolates resistant to the indicator carbapenem by clinical breakpoint or otherwise positive by the EUCAST screening criteria (see section 7) should be subjected to confirmatory tests[41].

Perform a molecular or immunochromatographic assay for at least the detection of KPC, OXA-48-like, NDM and VIM carbapenemase families on isolates.

If required, perform carbapenemase confirmatory tests as recommended by EUCAST on isolates found resistant or to have reduced susceptibility to the indicator carbapenem.

EUCAST advocate that supplemental tests to confirm carbapenemase production are unnecessary for individual patient management; the only test needed is MIC determination – either by agar or broth dilution, or by use of gradient strip methods[6,59]. The risk of onward spread may vary with underlying resistance mechanisms. EUCAST indicate the value of supplemental testing for infection prevention and control purposes, and for local epidemiological investigations.

7.2 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

7.2.1 Controls for carbapenemase tests

Quality control of the carbapenem discs used in the screening should follow standard EUCAST recommendations.

Positive controls should be used to ensure the performance of carbapenemase confirmatory tests. Various strains, including strains that express known carbapenemases and EUCAST recommended control strains are available from Public Health England's National Collection of Type Cultures (NCTC) (https://www.phe-culturecollections.org.uk/media.aspx?pid=182182). The NCTC online catalogue can be searched using the NCTC numbers listed below. Alternatively, some may be obtained commercially from other suppliers.

Table 3. Selected control strains producing carbapenemases available from the NCTC

Class A Carbapenemases		
Organism	**NCTC strain reference**	**Characteristics**
Klebsiella pneumoniae	NCTC 13438	Member of the international ST258 clone producing KPC-3 non-metallo-carbapenemase
	NCTC 14327	KPC-3 non-metallo-carbapenemase
	NCTC 14384	KPC-33 non-metallo-carbapenemase
Escherichia coli	NCTC 13919	GES-5 non-metallo-carbapenemase
	NCTC 14320	KPC non-metallo-carbapenemase IMP metallo-carbapenemase OXA-48-like non-metallo-carbapenemase

	NCTC 14321	KPC non-metallo-carbapenemase OXA-48-like non-metallo-carbapenemase
Serratia marcescens	NCTC 13920	SME-4 non-metallo-carbapenemase
Enterobacter cloacae / complex	NCTC 13922	NMC-A non-metallo-carbapenemase
	NCTC 13925	IMI-2 non-metallo-carbapenemase
	NCTC 14322	KPC-4 non-metallo-carbapenemase
	NCTC 14336	KPC-2 non-metallo-carbapenemase
Enterobacter asburiae	NCTC 14055	FRI-2 non-metallo-carbapenemase
Class B Carbapenemases (Metallo-β-lactamases)		
Organism	**NCTC strain reference**	**Characteristics**
Pseudomonas aeruginosa	NCTC 13437	VIM-10 metallo-carbapenemase; VEB-1 ESBL
	NCTC 13921	SPM-1 metallo-carbapenemase
	NCTC 14361	SIM metallo-carbapenemase
Pseudomonas guariconensis	NCTC 14056	DIM-1 metallo-carbapenemase
Klebsiella pneumoniae	NCTC 13439	VIM-1 metallo-carbapenemase
	NCTC 13440	VIM-1 metallo-carbapenemase
	NCTC 13443	NDM-1 metallo-carbapenemase
	NCTC 14323	NDM-1 metallo-carbapenemase; OXA-48 non-metallo-carbapenemase
	NCTC 14331	NDM-1 metallo-carbapenemase

	NCTC 14332	NDM-1 metallo-carbapenemase; OXA-232 non-metallo-carbapenemase
	NCTC 14334	IMP-4 metallo-carbapenemase
	NCTC 14337	IMP-1 metallo-carbapenemase
Escherichia coli	NCTC 13476	IMP-type metallo-carbapenemase
	NCTC 14320	IMP metallo-carbapenemase; KPC non-metallo-carbapenemase; OXA-48-like non-metallo-carbapenemase
	NCTC 14325	NDM-7 metallo-carbapenemase
	NCTC 14333	NDM-4 metallo-carbapenemase
	NCTC 14339	NDM-5 metallo-carbapenemase
Salmonella Seftenberg	NCTC 13953	NDM-1 metallo-carbapenemase
Enterobacter cloacae	NCTC 14326	VIM-1 metallo-carbapenemase
	NCTC 14328	VIM-4 metallo-carbapenemase
Citrobacter freundii	NCTC 14089	GIM-1 metallo-carbapenemase
Class D Carbapenemases (OXA carbapenemases)		
Organism	**NCTC strain reference**	**Characteristics**
Acinetobacter baumannii	NCTC 13301	OXA-23-like (and intrinsic OXA-51-like)
	NCTC 13302	OXA-25-like (OXA-24/40-like) (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13303	OXA-26 (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13304	OXA-27 (and intrinsic OXA-51-like) non-metallo-carbapenemases

	NCTC 13305	OXA-58-like (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13420	OXA-51-like non-metallo-carbapenemase
Klebsiella pneumoniae	NCTC 13442	Sequence type 353 with OXA-48-like
	NCTC 14323	OXA-48 non-metallo-carbapenemase; NDM-1 metallo-carbapenemase
	NCTC 14330	OXA-181 non-metallo-carbapenemase
	NCTC 14332	OXA-232 non-metallo-carbapenemase; NDM-1 metallo-carbapenemase
	NCTC 14335	OXA-232 non-metallo-carbapenemase
Escherichia coli	NCTC 14320	OXA-48-like non-metallo-carbapenemase; IMP metallo-carbapenemase; KPC non-metallo-carbapenemase9
	NCTC 14321	OXA-48-like non-metallo-carbapenemase; KPC non-metallo-carbapenemase
	NCTC 14324	OXA-484 non-metallo-carbapenemase
	NCTC 14329	OXA-244 non-metallo-carbapenemase
	NCTC 14338	OXA-48 non-metallo-carbapenemase
Salmonella Typhimurium	NCTC 13954	OXA-48 non-metallo-carbapenemase, as mediated by the pOXA-48a-like plasmid

Note: Either *Escherichia coli* NCTC 10418 or NCTC 12241 (equivalent to ATCC 25922) should be used as a negative control in confirmation tests.

A lenticule® disc including NCTC positive controls for the 'big 5' carbapenemases is available from NCTC (https://www.phe-culturecollections.org.uk/products/bacteria/antimicrobial-resistance-gene-controls.aspx). To assist with local validation of in-house or commercial molecular and

immunochromatographic assays a panel of CPE isolates (NCTC 14320 – NCTC 14339 consecutively, and listed above) representing common variants of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases known to be circulating in the UK can also be obtained from the NCTC.

8. Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see PHE user manuals and request forms.

Frontline diagnostic laboratories are strongly recommended to implement a PCR or immunochromatographic assay for detection of the 'big 4' carbapenemase families[58] in any isolate that appears to be resistant to the indicator carbapenem. Some PHE Specialist Laboratories offer referral services at a regional level, and then refer selected isolates onwards to the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit in Colindale. Laboratories using this regional service should not submit isolates to AMRHAI directly. All carbapenemase producers confirmed by diagnostic laboratories or PHE Specialist Laboratories from normally sterile sites only should be referred to AMRHAI for inclusion in the national strain archive. Refer to PHE's Bacteriology Reference Department user manual for up-to-date guidance on bacterial isolates that should be referred to the AMRHAI Reference Unit, turnaround times, transport procedure and the other requirements.

From 1 October 2020, diagnostic laboratories in England will have the duty to report the following to PHE:

- Acquired carbapenemase-producing Gram-negative bacteria isolated from human samples
- The results of any antimicrobial susceptibility test and any resistance mechanism identified in any of the causative agents listed in Schedule 2 of the Regulations.

Contact the appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit
Bacteriology Reference Department
National Infection Service
Public Health England
61 Colindale Avenue
London
NW9 5EQ

https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services

Telephone: +44 (0) 208 3277887

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Wales

Specialist Antimicrobial Chemotherapy Unit
Public Health Wales Microbiology Cardiff
University Hospital of Wales
Heath Park
Cardiff

CF14 4XW

Specialist Antimicrobial Chemotherapy Unit (SACU) 2021 - Public Health Wales (nhs.wales)

Scotland

https://www.hps.scot.nhs.uk/a-to-z-of-topics/reference-laboratories/#publications

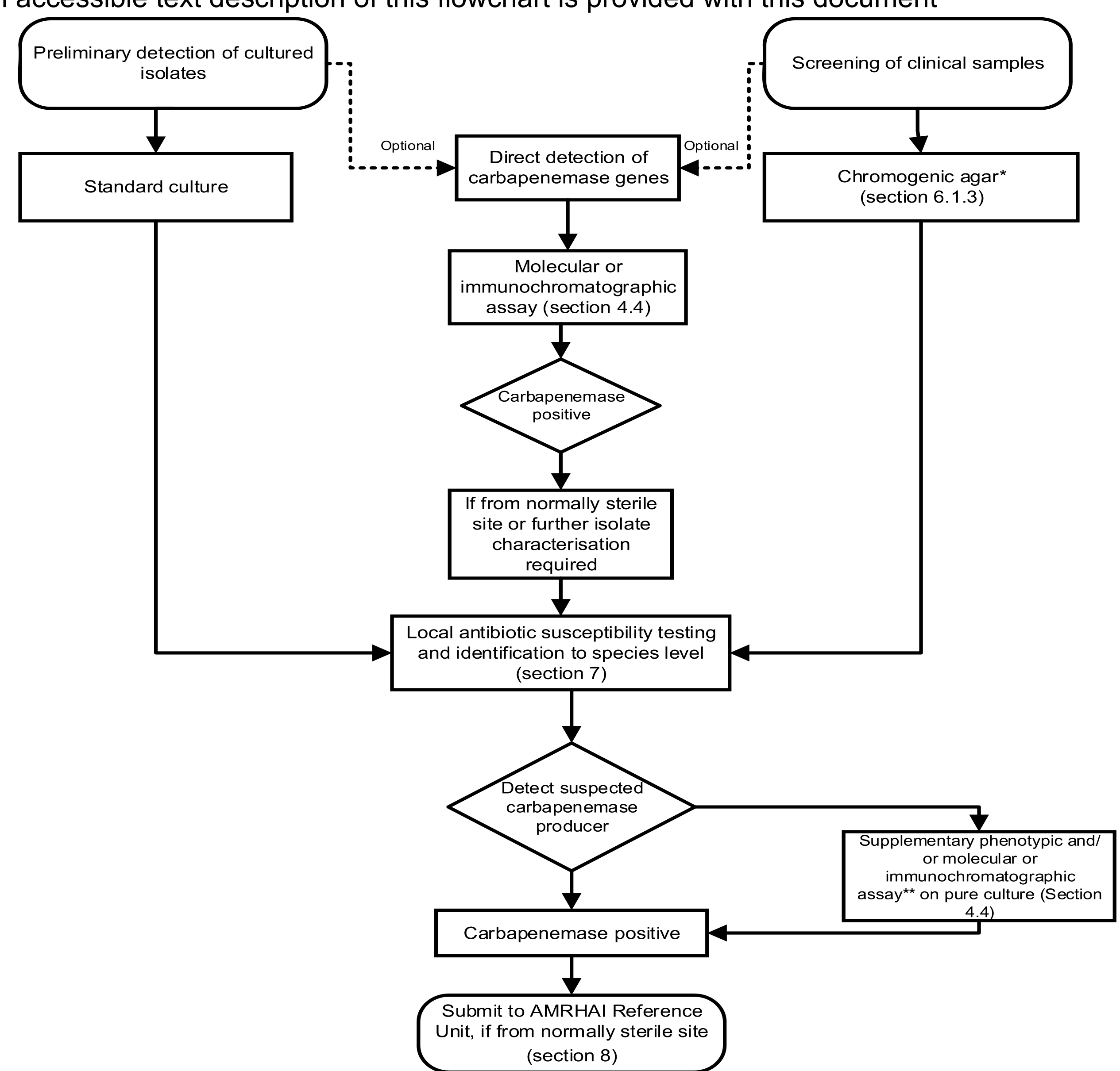
Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix 1: Flowchart for the detection of carbapenemases on cultured isolates and screening samples

An accessible text description of this flowchart is provided with this document



*If not available use MacConkey / CLED agar + 10µg ETP disc.
**As a minimum perform molecular or immunochromatographic detection of the 'big 4' (KPC, OXA-48-like, NDM and VIM). Isolates that are negative for the 'big 4' carbapenemase families should also be referred to the AMRHAI Reference Unit to rule out presence of rarer carbapenemase families.

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UK Standards for Microbiology Investigations

Investigation of specimens for ectoparasites

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

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Bacteriology | B 61 | Issue no: 2.2 | Issue date: 12.10.18 | Page: 1 of 62

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UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

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Contents

Acknowledgments 2

Amendment table 4

UK SMI: scope and purpose 6

Scope of document 9

Key recommendations 9

Introduction 9

Technical information/limitations 51

1 Safety considerations 53

2 Specimen collection 54

3 Specimen transport, storage and retention 55

4 Specimen processing/procedure 55

5 Reporting procedure 57

6 Notification to PHE, or equivalent in the devolved administrations 57

References 59

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	8/12.10.18
Issue number discarded	2.1
Insert issue number	2.2
Anticipated next review date*	27.04.18
Section(s) involved	Amendment
Whole document.	References have been converted from Reference Manager to Endnote.

Amendment number/date	7/27.10.16
Issue number discarded	2
Insert issue number	2.1
Anticipated next review date*	27.04.18
Section(s) involved	**Amendment**
Whole document.	B 61 formerly G 9. Document presented in a new format.

Amendment number/date	6/27.04.15
Issue number discarded	1.4
Insert issue number	2
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	Minor text changes for clarity. Minor grammatical corrections.

Section 3 – fleas.	Photographs and illustrations of *Ctenocephalides canis* updated. Information regarding *Ceratophyllus* and *Nasophyllus* species expanded.
Section 4 – lice.	Addition of illustration of female *Pediculus humanus.*
Section 5 – bedbugs.	Photographs and illustrations of *Cimex lectularis* updated.
Section 10 - laboratory procedures.	Under section 10.1 Safety Considerations – addition of text regarding processing of samples where Hazard Group 3 organisms are suspected.
References.	Some references updated.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested citation for this document

Public Health England. (2018). Investigation of specimens for ectoparasites. UK Standards for Microbiology Investigations. B 61 Issue 2.2. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

Type of specimen

Ectoparasitic arthropods, leeches

This UK SMI describes the examination of samples for ectoparasites.

This UK SMI should be used in conjunction with other UK SMIs.

Key recommendations

Not applicable.

Introduction

This UK SMI deals with the most important human ectoparasites (the fleas, lice, bedbugs, ticks and mites). Myiasis flies are also included in this UK SMI although only a few species are obligate parasites of mammals. Descriptions and diagrams are included to distinguish ectoparasites commonly found in the UK and those associated with foreign travel. The parasites covered in this UK SMI are rare laboratory specimens in the UK and for this reason it is considered advisable that only experienced personnel should examine them. Identification is subjective and therefore should be checked by a second person and confirmed by a reference laboratory.

It is important to ascertain whether an ectoparasitic infection is the cause of a patient's symptoms or a potential vector of disease. Ectoparasites are recognised by their size, colour, and morphological appearance. Reference to an appropriate ectoparasite identification key may aid identification[1].

The arthropod ectoparasites are structurally unrelated and form a biological rather than a taxonomic group. Most parasitic arthropods are ectoparasites (that is, they live and feed on the outside of their host) but certain species are more intimately associated with their human and animal hosts. The morphology of fleas, lice, bedbugs, ticks and mites shows several features adapted for an ectoparasitic life. These include the absence of wings, presence of spines and bristles, terminal claws on legs for clinging to fur/feathers of hosts, and a tough chitinous body. These features reduce the risk of dislodgement of the parasite by the host as there may be intense host grooming or scratching in response to any infestation.

The biology of ectoparasite vectors

In general, fleas, bedbugs, ticks and mites of medical importance are not host specific. These ectoparasites are primarily zoophilic but are opportunistic feeders on man.

In contrast, lice are highly host specific and spend their entire life cycle on the host. Body lice are transferred by close physical contact or the exchange of infested clothing. They are vectors of classical epidemic typhus, louse-borne relapsing fever and quintana fever.

All these ectoparasites may require a single large blood meal or regular small blood meals to complete their life cycles. In addition to disease transmission their bites cause lesions in the skin that may prove slow to heal, or may become secondarily infected by bacteria. The role of faeces and the bodies of the ectoparasites themselves in causing allergic responses should also be mentioned, as asthma, dermatitis and allergic rhinitus are becoming increasingly prevalent.

Disease transmission

Ectoparasites make extremely good vectors of disease because they spend extended periods of time in contact with the host. Transmission may be due to inoculation via saliva as in mite-borne scrub typhus; inoculative via regurgitation, as in plague; contamination by faeces, as in louse-borne typhus; contamination by secretion, as in tick-borne relapsing fever; or contamination by crushed vector, as in louse-borne relapsing fever.

Fleas, ticks and mites transmit a variety of animal pathogens and the diseases they pass to man are primarily zoonoses. Arthropod ectoparasites are vectors of viral (arboviruses), rickettsial (typhus fevers), bacterial (relapsing fever, plague) and protozoal (babesiosis, East Coast fever) infections. Most of the diseases are worldwide in distribution and many, such as plague and tick-borne or mite-borne infections are localised, forming restricted foci not involving man. There is no evidence that bed-bugs are significant vectors of human pathogens.

The transmission of pathogens by ectoparasites can occur in several ways and of these trans-stadial and trans-ovarial transmission are of particular interest. Trans-stadial transmission occurs when a pathogen is maintained in a vector throughout its life stages (that is, acquired as a larva, passed on to the nymph and passed on to the adult). In trans-ovarial transmission the pathogen is passed on to the next generation through the egg. These are both examples of vertical transmission through the vector, population and in such circumstances a disease can be maintained in an area without passing through a human host.

Fleas[2]

Fleas (order: Siphonaptera) are true insects (class: Insecta) and as such have a segmented body that is clearly divided into head, thorax and abdomen. In the adult stage the thorax bears six legs.

Adult fleas are obligate parasites of vertebrate animals. In general they are not host specific and will take a blood meal from any available host when hungry. However, for the female flea to successfully develop her eggs she depends on blood meals taken from the primary host. Only a small number of the many species of fleas are of any medical importance and it should be noted that diseases transmitted by fleas are

zoonoses (diseases of animals). Fleas must therefore have access to both human and animal populations if they are to act as vectors of disease.

Description

Fleas are small (1-8mm long), oval, wingless insects that vary in colour from yellowish brown to black. The body is flattened laterally, has a shiny, waxy cuticle and bears numerous stout spines and bristles. Most species have one pair of well-developed eyes and clubbed antennae tucked into folds behind the eyes. Some fleas possess combs; finger-like outgrowths of cuticle around the mouth (genal comb) or as a collar on the first thoracic segment (pronotal comb). A rod in the second thoracic segment (a mesopleural rod) is seen in some species.

Lifecycle

Fleas exhibit a complete metamorphosis; the immature stages do not resemble the adult and occupy very different ecological niches. On average an adult flea lives for 6-12 months and has been postulated to live for 2 years. If a female flea has access to the primary host she can mature eggs and may lay 300-1000 eggs over her lifetime (averaging 3-25 a day). The eggs hatch in 2-14 days and caterpillar-like larvae emerge. Flea larvae are elongate, have no legs and are sparsely covered with long setae. They have small heads with simple antennae but lack eyes.

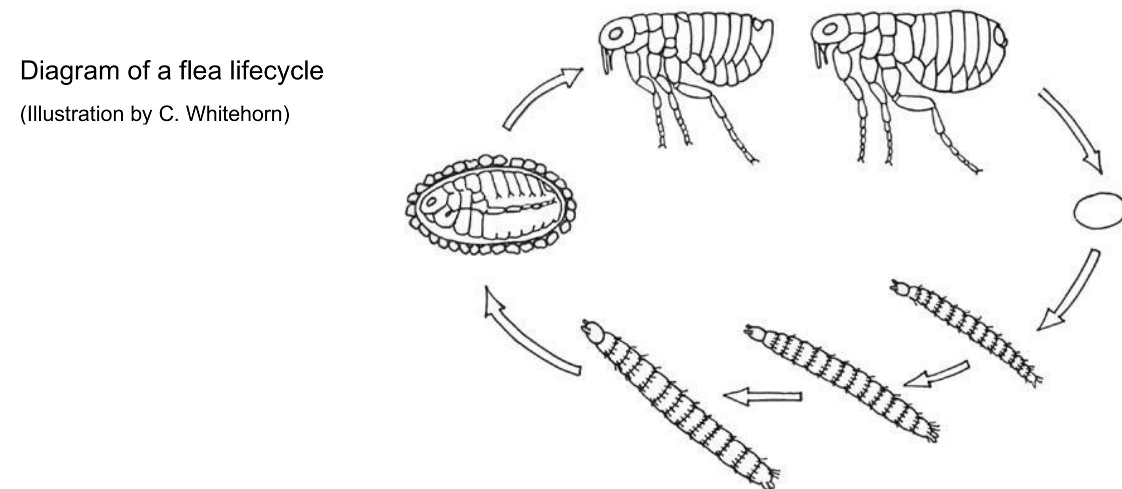


Diagram of a flea lifecycle

(Illustration by C. Whitehorn)

The larval abdomen bears one pair of anal struts. The larvae feed on organic debris found in the nest (or house) of the host. The larval diet often includes partially digested blood passed in the faeces of adult fleas. The larvae undergo two moults and mature after 2 to 3 weeks. They then produce silk and spin a cocoon within which they undergo a further moult and become a pupa. The pupal stage lasts for 1 to 2 weeks; the adult flea then sheds the pupal skin and remains dormant within the cocoon until triggered to emerge by specific stimuli. Adult fleas can copulate immediately after emergence and egg production can begin within 1 to 2 days of obtaining a blood meal.

Pathology of flea bites

When a flea feeds it injects saliva into the dermis to prevent the blood meal from coagulating. This causes an intense itching at the bite site which lasts for several days. Typically hypersensitivity develops in people exposed to repeated flea bites. A flea bite is characterised by a tiny dark spot surrounded by reddish and swollen skin.

Medically important fleas

The species which have the greatest impact on man are *Xenopsylla cheopis* (the plague flea), *Pulex irritans* (the human flea) and *Tunga penetrans* (the jigger flea). There are also a number of animal and bird fleas that will feed opportunistically on man and may cause a severe biting nuisance. Refer to Lane and Crosskey for keys to the medically important genera and species of fleas[1].

Preparation of material

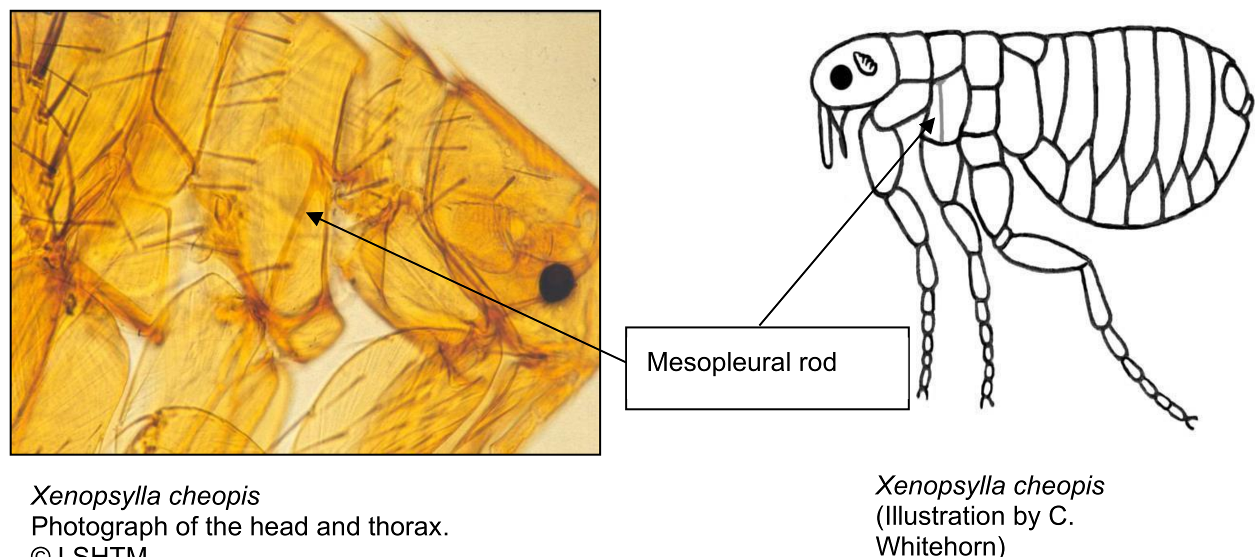
Adult fleas should be killed and stored in 80% ethanol prior to preparation. Transfer the flea to a watch glass or similar container containing 10% potassium hydroxide (KOH) solution for 24 hours, or longer, until the body contents are clear. Transfer the flea directly to a watch glass or similar container containing glacial acetic acid for a minimum of 2 hours. Then transfer the flea to clove oil for 2-24 hours until the body cavity is clear and the genitalia are visible. Mount the whole specimen in Euparal and add a coverslip. With care the specimen can be examined immediately. The specimen should be placed in an oven for 4-6 weeks at 55°C to give a permanent preparation. Label the slide with the identification, reference number and collection data.

Xenopsylla cheopis (plague or tropical rat flea)

Cosmopolitan in distribution, the flea is principally an ectoparasite of rats. It is a vector of plague and murine typhus.

Description

The flea is distinguished from other genera by the absence of both a genal and a pronotal comb, and the possession of a mesopleural rod within the second thoracic segment.



Xenopsylla cheopis
Photograph of the head and thorax.
© LSHTM

Xenopsylla cheopis
(Illustration by C. Whitehorn)

Plague

Plague is a bacterial infection caused by *Yersinia pestis*. It is a zoonotic infection maintained in wild rodent populations (sylvatic plague) that is occasionally transmitted to commensal rats (urban plague). Commensal rats have less resistance to the disease and die in large numbers. The fleas that were living on them then seek alternative hosts. *Y. pestis* taken from an infected host in a blood meal undergoes a

rapid multiplication in the stomach of the flea. The bacteria form a viscous plug that blocks the stomach and prevents the flea from feeding normally. When the flea attempts to feed, the blood comes up against the plug of bacteria and cannot enter the stomach. It is instead regurgitated by the flea back into the host and takes some of the bacteria with it, infecting the new host.

Murine typhus

Murine typhus is a rickettsial infection caused by *Rickettsia mooseri*. It is a zoonotic disease of rats and mice. The rickettsiae are ingested from an infected host with the blood meal. There is a multiplication of rickettsiae in the gut of the flea but no blockage occurs and the infective stages are passed out in the faeces. Transmission occurs when faeces from infected fleas are scratched into the skin, rubbed into mucous membranes or inhaled. Ingestion of contaminated fleas is also a route of infection.

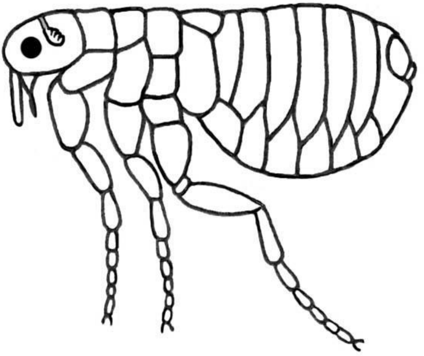
Pulex irritans (human flea)

Cosmopolitan in distribution, the flea is principally an ectoparasite of coarse-coated mammals such as pigs, boar and deer, but also occurs on humans. It is mainly important as a biting nuisance, but is a vector of plague in the USA.

Description

The flea is distinguished from other genera by the absence of both a genal and a pronotal comb, and the presence of a thickened interantennal suture between the antennae. *P. irritans* does not have a mesopleural rod.

Pulex irritans
Photograph of head and thorax
© LSHTM



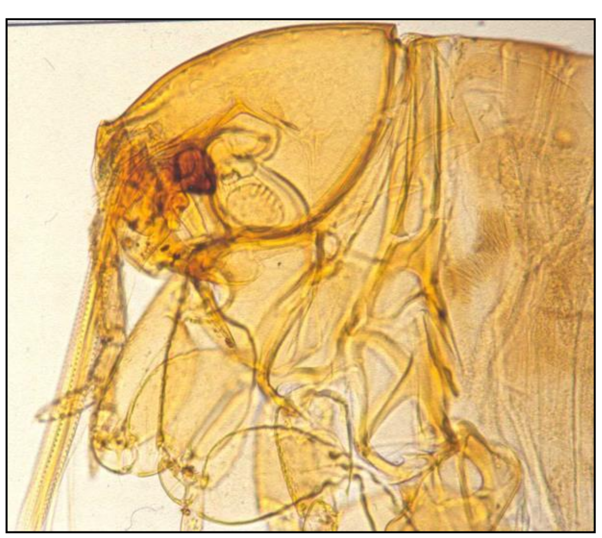
Pulex irritans
(Illustration by C. Whitehorn)

Tunga penetrans (the jigger flea)[3]

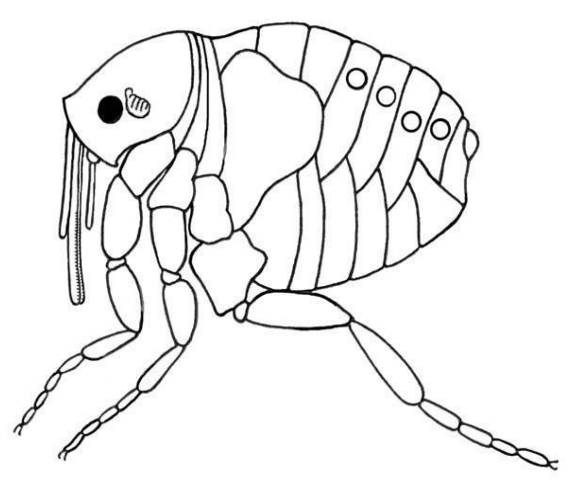
Distributed throughout tropical Africa, South and Central America, this flea is an ectoparasite of man, domestic livestock and rodents. Infestations of this flea cause the condition known as tungiasis; the female flea burrows into the skin of the host and becomes permanently attached.

Description

This type of flea is distinguished from other genera by its small size (1mm), the lack of a genal and pronotal comb, the compressed thoracic segments and the distinctive shape of the head.



Tunga penetrans
Photograph of head and thorax.
© LSHTM



Tunga penetrans
(Illustration by C. Whitehorn)

Tungiasis

Both male and female jigger fleas feed on blood, but the male flea will leave the host after taking a blood meal. The female flea once fertilised burrows into the skin of the host until she becomes totally embedded with only the tip of the abdomen exposed. Favoured sites include the foot, the toes and under the toenails, but any part of the body may be affected. As the female digests her blood meal, the eggs mature and she swells to the size and shape of a small pea. The enlargement results in considerable discomfort for the host. After 8-10 days the female has attained her maximum size and mature eggs are shed from the genital opening. Approximately 200 eggs are shed over a one to two week period. The eggs fall to the ground and hatch after 3-4 days. The larvae and pupae are found in sandy, well-drained soils. With larval development taking 10-14 days and the pupal stage 5-14 days, the entire lifecycle is completed in 35 days on average. When the female flea dies she remains embedded in the skin, causing inflammation that may lead to secondary infections. Loss of toes, tetanus and gangrene may occur.

Animal and bird fleas[2]

A number of animal and bird fleas will bite man opportunistically and can cause considerable biting nuisance. These fleas can also act as intermediate hosts of animal tapeworms. The most important genera are *Ctenocephalides* (cat and dog fleas) and *Ceratophyllus* (bird fleas), and to a lesser extent *Nosopsyllus* (rat fleas) and *Leptopsylla* (mouse fleas)[1].

Description

The animal and bird fleas all possess a pronotal comb. The number of spines present in the pronotal comb is important for identification. *Leptopsylla* species and *Ctenocephalides* species also possess a genal comb.

Ctenocephalides felis (the cat flea)

Cosmopolitan in distribution, *C. felis* is an ectoparasite of cats and dogs, and is the flea that most commonly bites man in the UK. It can be distinguished from the dog flea

C. canis by the elongate head of the adult and the arrangement of setae on the hind tibia[1]. *C. felis* can act as the intermediate host of the dog tapeworm *Dipylidium caninum* and the mouse tapeworm *Hymenolepis diminuta.*

Description

The cat flea is distinguished from other genera by the presence of a genal comb (of 7 to 8 points), a pronotal comb and the presence of a mesopleural rod. The head of the cat flea is twice as long as high and is pointed anteriorly. The hind tibia has six seta-bearing notches along the dorsal margin[1].

Ctenocephalides felis

Photograph of head, first and second thoracic segments. **Note:** combs visible.

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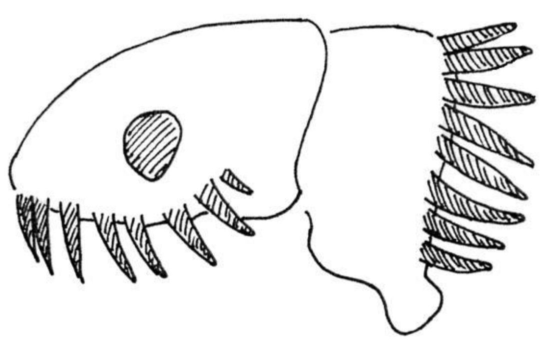
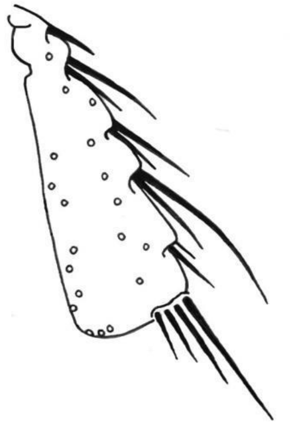


Ctenocephalides felis

Drawing of head and first thoracic segments.

(Illustration by C. Whitehorn)



Ctenocephalides felis

Drawing of hind tibia.

(Illustration by C. Whitehorn)

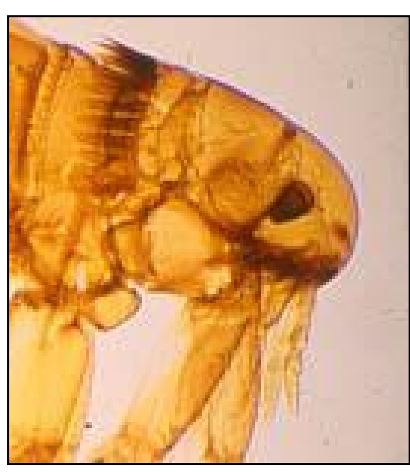
Note: Circles denote the origin of additional setae that are not illustrated.

Ctenocephalides canis (the dog flea)

Cosmopolitan in distribution, *C. canis* is found on both cats and dogs, and will also bite man. *C. canis* is less common in the UK than the cat flea. The dog flea is distinguished from the cat flea by its rounded head and the arrangement of setae on the hind tibia. *C. canis* can act as the intermediate host of the dog tapeworm *Dipylidium caninum*.

Description

The dog flea is distinguished from other genera by the presence of a genal comb (of 7 to 8 points), a pronotal comb and the presence of a mesopleural rod. The head of *C. canis* is rounded anteriorly and its length is up to twice that of its height. The hind tibia has eight seta-bearing notches along the dorsal margin[1].



Ctenocephalides canis

Photograph of head, the first and second thoracic segments.

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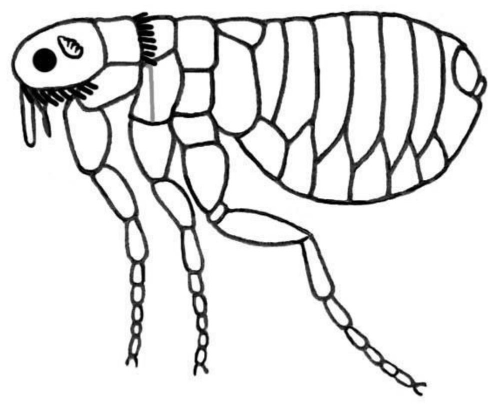


Ctenocephalides canis

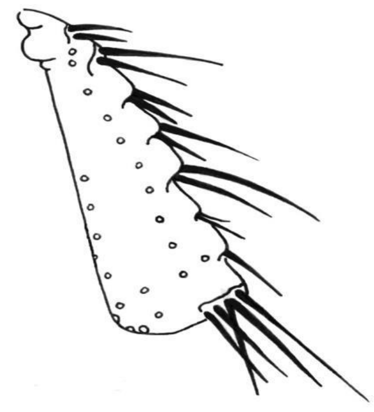
(Illustration by C. Whitehorn)

Ctenocephalides canis

Drawing of hind tibia.

(Illustration by C. Whitehorn)

Note: Circles denote the origin of additional setae that are not illustrated



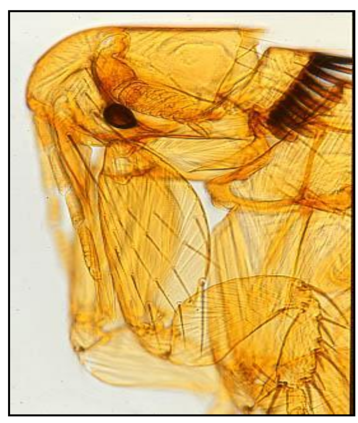
Ceratophyllus (the bird flea) and *Nosopsyllus* (the rat flea)

Both these genera are found in the family Ceratophyllidae and share a number of morphological characters. They can be distinguished only by examination of the genitalia. The common chicken flea (*Ceratophyllu*s *gallinae*) is cosmopolitan in distribution and is an ectoparasite of domestic poultry and wild birds (such as pigeons, starlings and sparrows). This species will bite man opportunistically and causes

considerable biting nuisance. The rat flea (*Nosopsyllus* species) is also cosmopolitan in distribution and is the ectoparasite of a number of rodent species.

Description

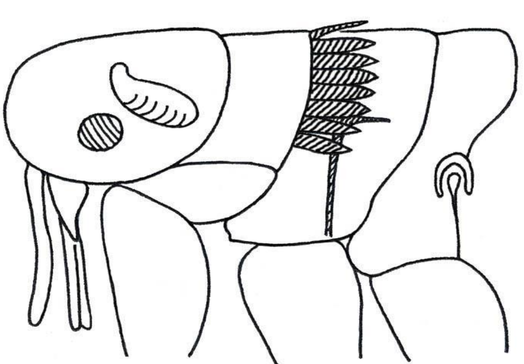
These fleas are distinguished from the other medically important genera by the presence of a pronotal comb, a mesopleural rod and a well-developed pleural arch (located between the third thoracic segment and the abdomen).



Nosopsyllus species

Photograph of head, first and second thoracic segments.

© LSHTM.



Nosopsyllus species

Drawing of head and thorax.

Note: pleural arch in third thoracic segment.

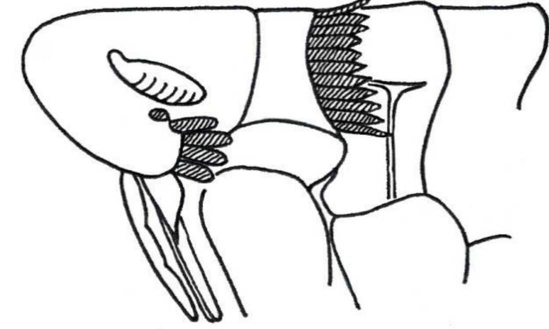
(Illustration by C. Whitehorn)

Leptopsylla (the mouse flea)

Distributed throughout the Palaearctic, Nearctic and Afrotropical regions members of the genus *Leptopsylla* are primarily ectoparasites of small rodents.

Description

These fleas are distinguished from other genera by the distinctive shape of the head (that appears folded), the presence of a pronotal comb and a reduced genal comb, and the absence of eyes.



Leptopsylla species

Drawing of head, first, second and part of the third thoracic segment.

(Illustration by C. Whitehorn)

Table 1: Fleas associated with man

Genus	Host	Head	Genal-comb	Pronotal-comb	Eyes	Mesopleural rod
Pulex	Human and other mammals	Normal	Absent	Absent	Present	Absent
Xenopsylla	Rats	Normal	Absent	Absent	Present	Present
Tunga	Human and domestic animals	Upturned	Absent	Absent	Present	Absent
Ctenocephalides	Cat/Dog	Normal	Present	Present	Present	Present
Ceratophyllus	Birds	Normal	Absent	Present	Present	Present
Nosopsyllus	Rats	Normal	Absent	Present	Present	Present
Leptopsylla	Mice	Folded	Present	Present	Absent	Present

Ceratophyllus and Nosopsyllus also have a pleural arch located between the third thoracic segment and the abdomen.

Lice[2,4]

Human lice

Lice (order: Anoplura) are true insects (class: Insecta) and as such have a segmented body that is clearly divided into head, thorax and abdomen. In the nymph and adult stages the thorax bears six legs.

Lice are obligate parasites of vertebrate hosts throughout their lifecycle. They are host specific and may even exhibit region specific behaviour on that host. There are three species of lice found on man and of these only one, *Pediculus humanus*, is a vector of disease.

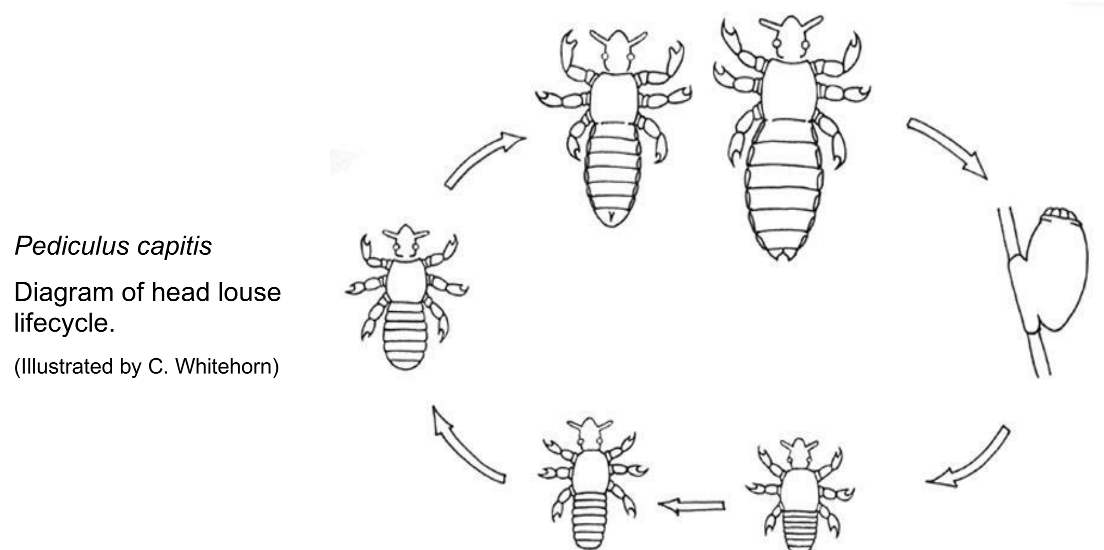
Description

Human lice are small (2-4mm long), wingless insects that vary in colour from cream to dark brown depending on their host. The body is flattened dorso-ventrally and is covered with a leathery integument. The body is clearly divided into head, thorax and abdomen but the thorax and abdomen are fused. The head bears a pair of small eyes and one pair of short antennae. The thorax bears three pairs of legs with strong claws for clinging to the host. The human lice are classified in two genera; *Pediculus* species (the clothing and head lice) and *Phthirus* species (the crab louse).

Lifecycle[5,6]

Lice exhibit an incomplete metamorphosis with the immature stages resembling the adult and occupying the same ecological niche. They spend their entire lifecycle on the host and depart only to transfer to a new host. Adult head lice live on average 22 days and the females lay about 50 eggs over their lifetime. Adult clothing lice live on average 30 days and the females lay about 100 eggs over their lifetime. Adult crab lice live for approximately 22 days and the females lay about 50 eggs over their lifetime. Eggs hatch after 6-8 days in all cases (at 35°C) and a small nymph emerges. There

are 3 nymph stages. For the head and clothing lice, nymph development takes approximately 9 days to complete. For the crab louse, nymph development takes 15-17 days. Human lice need to take regular feeds throughout the day and are very sensitive to changes in temperature and humidity.



Pediculus capitis

Diagram of head louse lifecycle.

(Illustrated by C. Whitehorn)

Pathology of lice bites[5,6]

Lice are equipped with discrete mouthparts contained in a ventral pouch. When a louse feeds it attaches to the skin using a toothed haustellum and pierces the skin with needle-like stylets. Saliva is injected into the wound to prevent coagulation and the blood is sucked up through a flexible tube-like mouth. The bites of human lice result in small red spots 2-3mm across. Sensitivity to lice bites may develop over a period of weeks or months (depending on the level of exposure) and once established the skin irritation may be severe. The need for lice to take regular feeds means that the host will be exposed to repeated doses of saliva and a toxic reaction may occur in some individuals with symptoms of weariness, irritability and depression (the person feels 'lousy').

Medically important lice

Only the clothing louse *Pediculus humanus* is a vector of disease, transmitting louse-borne epidemic typhus, quintana fever and louse-borne relapsing fever. However the regular feeding habits of lice mean that severe biting nuisance is associated with all three species.

Preparation of material

Lice should be killed in hot water (85°C) and stored in 80% ethanol prior to preparation. Transfer the louse into a watch glass on similar container containing 10% potassium hydroxide (KOH) solution. Pierce the dorsal inter-segmental membranes with a fine needle and leave the louse in the solution for 24 hours. The KOH penetrates and dissolves the body tissues. Apply gentle pressure to the body of the louse with a blunt needle to remove the liquefied body contents. Rinse the louse in distilled water three times (10 minutes per rinse). Dehydrate in increasing strengths of ethanol 80% (5 mins), 90% (5 mins), 100% (5 mins) and finally place in a watch glass on similar container containing cellosolve for 15 minutes. Mount the whole specimen in Euparal and carefully add a coverslip. With care the specimen may be examined immediately. The specimen should be placed in an oven for 4-6 weeks at 55°C to give

a permanent slide preparation. Label the slide with the identification, reference number and collection details of the specimen.

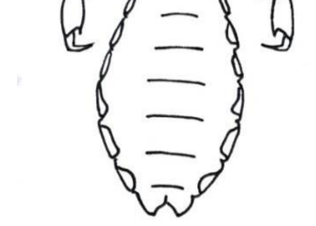
Pediculus Humanus (the clothing louse)

Cosmopolitan in distribution, the clothing louse is associated with displaced populations where people are unable to wash or change clothes frequently. The most robust of the human lice it can survive several days away from the host in infested clothing.

Description

Adult

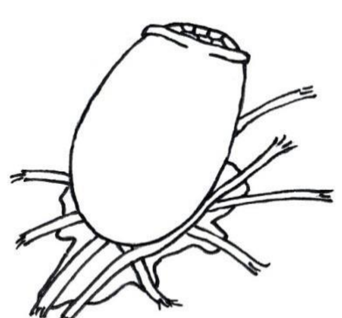
The human *Pediculus* species are elongate insects which grow to a length of about 4mm. The body regions are clearly differentiated and the legs bear claws of a moderate size. Females are slightly larger than males. Clothing lice and head lice are practically identical and are differentiated by their location on the host. Clothing lice are found in the fabric covering the body particularly in the seams around the crotch, armpits, waist, collar and shoulders. They attach to body hair only when feeding and are never found on the head.



Pediculus humanus. Diagram of a female clothing louse.

Note: Tip of abdomen bifurcated.

(Illustration by C. Whitehorn)



Pediculus humanus.

Diagram of egg glued to material fibres.

(Illustration by C. Whitehorn)

Egg

The eggs of *Pediculus* species are small (0.8mm long), cream in colour and oval in shape[7]. At the distal end is a shallow perforated operculum that provides gaseous exchange to the embryo. The eggs of clothing and head lice are so similar that their location on the host is the primary method of differentiating the two species. Clothing lice eggs are found in the clothing glued to material fibres, especially in the seams of undergarments. They are occasionally glued to body hairs, but clothing lice eggs are never found on the head.

Louse-borne epidemic typhus

Louse-borne typhus is a rickettsial disease caused by *Rickettsia prowazekii.* The rickettsiae are ingested from the host during a blood meal and undergo multiplication in the lumen and epithelial cells of the louse midgut. The epithelial cells eventually rupture, releasing infective stages which are passed out in the faeces. The rickettsiae remain infective to humans in the louse faeces for up to 3 months and transmission occurs when faeces are scratched into the skin, rubbed into mucous membranes or inhaled. People without lice can therefore become infected with typhus. *R. prowazekii* has also been isolated from the American flying squirrel, but the significance of this for transmission is uncertain.

Quintana fever

Quintana fever is a bacterial disease caused by *Bartonella quintana*. The bacteria are ingested from the host during a blood meal and undergo multiplication in the lumen of the gut but the epithelial cells are not invaded. After 5-10 days the infective forms contaminate the faeces and they pass from the vector. The transmission route and symptoms are similar to louse-borne typhus but the disease is much less severe. Wild voles and other rodents may act as reservoirs of disease.

Louse-borne relapsing fever

The disease is caused by the spirochaete, *Borrelia recurrentis*. Humans are the sole reservoir of disease. The spirochaetes are ingested by the louse during feeding and penetrate the gut to multiply in the haemolymph. Transmission occurs when lice are ingested by humans or are crushed into abraded skin or crushed between the teeth. The disease is seldom seen in humans who are free of lice.

Pediculus capitis (the head louse)

Cosmopolitan in distribution, infestation is primarily seen in nursery and primary school children. Transmission of lice occurs during head to head contact. Lice do not survive if removed from the host.

Description

Adult

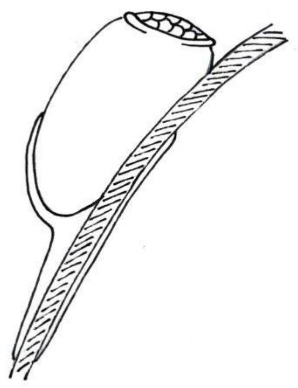
There are very few morphological differences between head and body lice but they can be distinguished by their location on the host. Head lice are only found on the hairs of the scalp.

Pediculus capitis - male
Photograph of the head and thorax
© LSHTM

Egg

The eggs of *Pediculus* species are small (0.8mm long), cream in colour and oval in shape[7]. At the distal end is a shallow perforated operculum that provides gaseous exchange to the embryo. The eggs of the head louse are differentiated from those of the body louse by their location, with head louse eggs restricted to the hairs of the scalp and no other part of the body.

Pediculus capitis
Photograph of head louse egg attached to strand of hair.
© LSHTM



Pediculus capitis – egg
Diagram of egg glued to strand of hair
(Illustrated by C. Whitehorn)

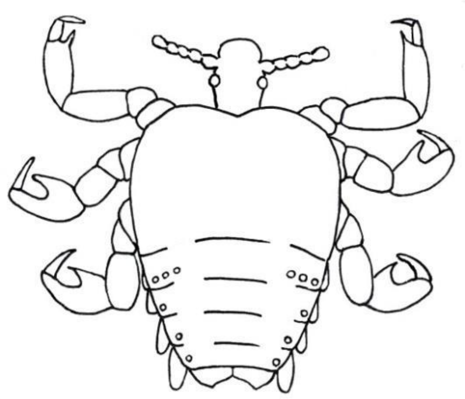
Phthirus pubis (the crab louse)

Cosmopolitan in distribution, infestation is primarily seen in sexually active adults. Infestation occurs on the coarse hairs of the body such as the pubic hair and eyelashes. In men the chest hair, beard and moustache may also be infested. Transmission of crab lice occurs during close physical contact.

Phthirus pubis

Photograph of crab louse – male

© LSHTM



Phthirus pubis

Diagram of crab louse – female

(Illustration by C. Whitehorn).

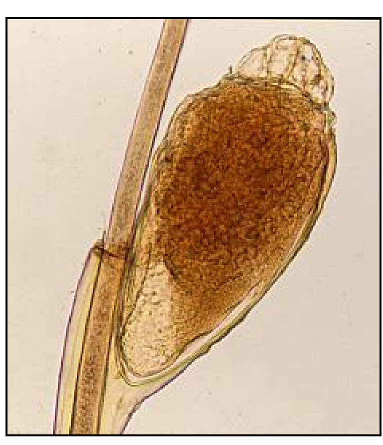
Description

Adult

Phthirus pubis is a compact rounded insect (1.0–1.4mm in diameter) with enlarged claws on the second and third pairs of legs. The abdomen is reduced both in size and number of segments, and there is no clear differentiation between the thorax and the abdomen. *Phthirus* resembles a tiny crab and hence its common name – the crab louse.

Egg

The eggs of *Phthirus pubis* are small (0.8mm long), cream in colour and oval in shape[7]. At the distal end is a raised perforated operculum that provides gaseous exchange to the embryo. The shape of the operculum differentiates the eggs of the crab louse from those of head and body lice.



Phthirus pubis – egg
Photograph of egg glued to strand of hair.

Note: raised operculum.

© LSHTM

Phthirus pubis – egg
Diagram of egg glued to strand of hair.
(Illustration by C. Whitehorn)

Although crab lice eggs may be found on the head of the host they are restricted to areas of coarse hair growth such as eyebrows, eyelashes, beards and moustaches. Crab lice eggs are not found on the hairs of the scalp.

Bedbugs[2]

Bedbugs (order: Hemiptera) are true insects (class: Insecta) and as such have a segmented body that is clearly divided into head, thorax and abdomen. In the nymph and adult stages the thorax bears six legs.

Bedbugs are obligate bloodsucking parasites of vertebrate animals. They are principally nocturnal and hide away in cracks and crevices during the day. Only two species are associated with man: *Cimex lectularius* (the common bedbug) and *Cimex hemipterus* (the tropical bedbug). Although these two species predominantly feed on man they may bite other mammals or birds. The medical importance of bedbugs is open to dispute, but they are responsible for considerable biting nuisance.

Photograph of *Cimex lectularius*

Male dorsal view.

© LSHTM

Photograph of *Cimex lectularius*

Female dorsal view.

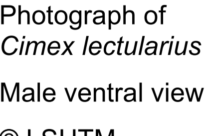
© LSHTM

Description

Adult bedbugs are small (5mm long by 3mm wide), oval insects that vary in colour from yellowish to dark brown and appear dark red if recently fed. The body is flattened dorso-ventrally and clearly divided into head, thorax and abdomen. The head is short and broad with one pair of eyes and one pair of 4-segmented antennae. Folded beneath the head is the proboscis (a 3-segmented rostrum) which is swung forward when the bug feeds. The thorax is divided into three segments and the shape of the

first segment (pronotum) is used to distinguish the common bedbug from the tropical bedbug. Dorsally the second and third thoracic segments are partially obscured by the two pad-like wings which are non-functional. The legs are well developed and bedbugs can move very quickly if disturbed.

The male can be distinguished from the female by examination of the abdomen. The male abdomen is narrower, slightly pointed and asymmetrical in outline. The female abdomen is rounded and symmetrical in outline.



Photograph of *Cimex lectularius*

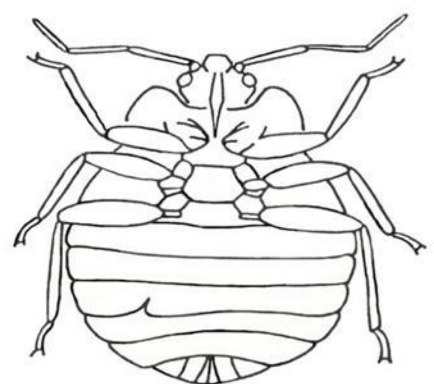
Male ventral view.

© LSHTM

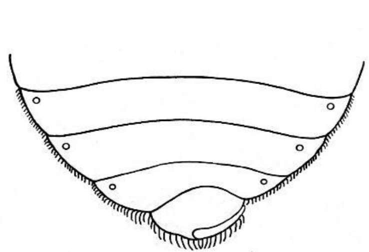
Cimex lectularius

Drawing of female bedbug ventral view.

(Illustration by C. Whitehorn)



In ventral view the terminal segment of the male bears a curved hook-like paramere (or penis), the external genitalia. In the female, the fourth abdominal segment bears a distinct slit to the left of the midline which opens into the copulatory pouch.



Cimex lectularius

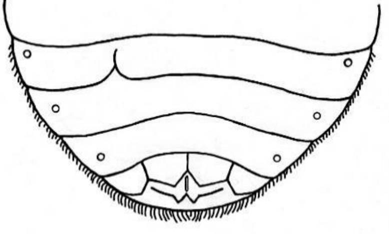
Drawing of male bedbug ventral view.

(Illustration by C. Whitehorn)

Photograph of *Cimex lectularius*

Close-up of male ventral view.

© LSHTM



Cimex lectularius

Drawing of female bedbug ventral view.

(Illustration by C. Whitehorn)

Photograph of *Cimex lectularius*

Close-up of female ventral view.

© LSHTM

Lifecycle

Bedbugs undergo an incomplete metamorphosis, and the immature and adult stages occupy identical ecological niches. Female bedbugs glue eggs in the cracks and

crevices of walls and furniture. Each female may lay 300 eggs over her lifetime depending on environmental conditions and access to blood meals. The eggs are elongate, cream to pink, operculate and approximately 1mm long. Eggs hatch after about 10 days and the first instar nymph emerges. There are 5 nymphal instars and each requires a blood meal to facilitate a moult to the next stage. Nymphs can survive for 4 months without a blood meal and adults can survive for over a year without feeding. The average time for completion of the lifecycle is 10 weeks but development is strongly influenced by temperature, humidity, host availability and habitat.

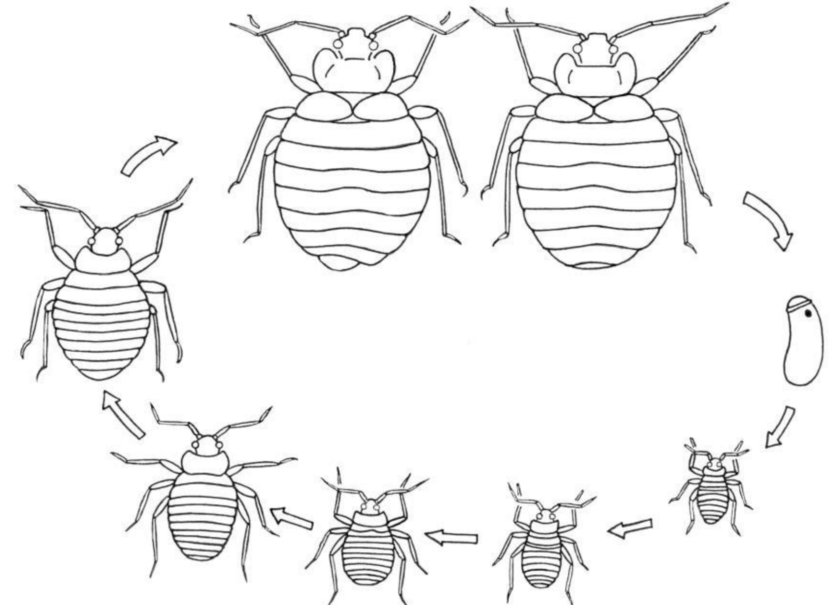


Diagram of the common bedbug lifecycle.

(Illustration by C. Whitehorn)

Signs and symptoms[8]

Bedbugs are believed to be transferred between houses within infested furniture and bedding. They are found across a broad range of premises from luxury properties to poor quality housing and predominantly in short-term accommodation such as hotels and hostels. Local dispersal of infestations can occur when bedbugs travel along the ducts, risers and central heating pipes that connect adjacent rooms. On average it can take 7 weeks for the infestation in one room to be detected in adjoining rooms. Such dispersal can occur even in the absence of any significant competition pressure. Adult bedbugs have scent glands that produce an odour when the bugs are disturbed. Properties with a heavy infestation may be identified by the musty, sweet, sweaty scent of this odour. Faecal spots around resting sites and the presence of cast skins would also indicate an infestation.

Reaction to bedbug bites varies considerably between individuals. Some people have no sensitivity to the saliva of bedbugs and are completely unaware that they are being bitten. However, in the majority of cases a localised itchy swelling will occur at the site of a bite approximately 15 to 30 minutes after feeding and may persist for several days. The interval between feeding and the occurrence of a reaction will depend on the host's immunological status. During the actual process of feeding the host normally feels no sensation at the bite site. Traditionally bites would occur on the head and shoulders of the person, as these were the exposed areas above close fitting bed sheets and blankets. A more diffuse feeding pattern may

Photograph of *Cimex* species feeding. © LSHTM

be seen with the use of loose fitting duvet covers or if the bugs are in other types of furnishings.

Medical importance

In the laboratory, bedbugs have been infected with hepatitis B, HIV and *Trypanosoma cruzi*, but there is little evidence to suggest that bed-bugs are significant vectors of human pathogens.

Preparation of material

Bedbugs should be killed by immersion in hot water (85°C) and then preserved in 70% ethanol. Bedbugs do not need to be slide mounted for identification, but simply placed into a watch glass or similar container containing 70% ethanol and examined under a dissecting microscope.

Cimex lectularius (the common bedbug)

In the UK the common bedbug is the predominant species responsible for domestic infestations. Over the last five years there has been a significant increase in cases but the reasons for this are unclear. Bedbugs continue to be associated with poverty and poor hygiene which makes frank discussion of this pest problematic.

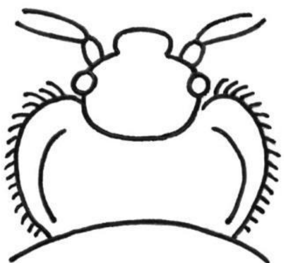
Description

Common bedbugs are approximately 4.5mm in length and 3mm in width. They are slightly smaller than the tropical bedbug and have a more rounded abdomen. Examination of the dorsal body surface shows that the pronotum (the first thoracic segment) is extended laterally into upturned flanges.

Cimex lectularius

Drawing of head and pronotum x 30.

(Illustration by C. Whitehorn)



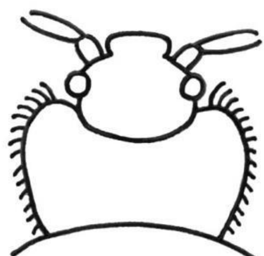
Cimex hemipterus (the tropical bedbug)

This species is common throughout the tropics and with the increase in international travel there was always the possibility that it might be introduced into the UK. There have been a small number of cases of domestic infestation by tropical bedbugs reported in recent years.

Description

Tropical bedbugs are approximately 5mm in length and 2.5mm in width. They are slightly larger than the common bedbug and have a slightly more elongated abdomen. Examination of the dorsal body surface shows that the pronotum (the first thoracic segment) is rounded and lacks lateral flanges.

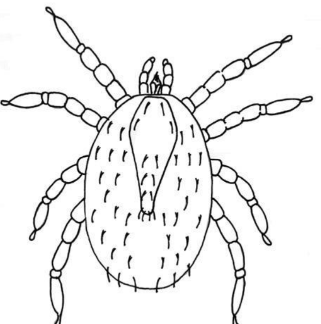
Cimex hemipterus
Drawing of head and pronotum x 30.
(Illustration by C. Whitehorn)

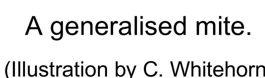


Mites[2]

Mites (subclass: Acari) are arachnids (class: Arachnida) and as such have a fused body that shows no division into head, thorax and abdomen. The body bears eight legs in the adult stages.

Mites are microscopic arachnids that occupy a diverse array of ecological niches. They are found in soil, air and water. They feed on plants, organic matter, other micro-organisms and occasionally vertebrates. The number of mites that are associated with man and that are of medical importance is extremely low. Mites typically require slide mounting and examination under a compound microscope for identification.





A generalised mite.
(Illustration by C. Whitehorn)

Description

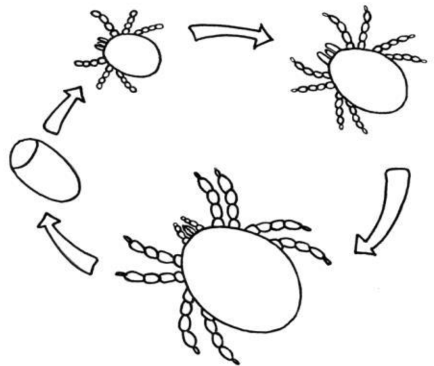
Mites are very small and normally range in size from 0.09–1.0mm in length (a few species can reach 15mm in length). The body is covered in a flexible cuticle that bears numerous setae often arranged in distinctive patterns. There is no division of the body into head, thorax and abdomen but the conspicuous mouthparts may be confused with a head. Mouthparts consist of a pair of palps and a pair of chelicerae (cutting/piercing apparatus). Adult and nymphal stages have eight legs whereas the larval stage has six legs. Larvae could thus be confused with insects but for their lack of body division. Mites may be differentiated from ticks by the following characters: mites have no prominent toothed hypostome in the mouthparts and no Haller's organ on the tarsi of the foreleg (see tick section).

Although mites are generally much smaller than ticks, size is not the best character for differentiating the two groups. Mites often have sclerotized regions on the body surface called shields and these, together with the setae that arise from them, are useful characters for identification.

Lifecycle

Mites demonstrate an incomplete lifecycle but the immature and adult stages may occupy widely different ecological niches. Lifecycles are given for each of the medically important mites in the appropriate section but a generalised lifecycle follows:

egg to six-legged larva, larva to eight-legged nymphal stage/s, nymph to adult. Female mites produce a small number of relatively large eggs which hatch to give the larva. After feeding the larva moults to give the nymph and the nymph may have up to three developmental stages (depending on species); protonymph, deutonymph and tritonymph. At least one nymphal stage is dormant. The nymph eventually moults to give the adult.



A simplified mite lifecycle.

(Illustrated by C. Whitehorn)

Medically important mites

The ectoparasitic mites of medical importance are *Sarcoptes scabiei* (scabies mite, itch mite), *Demodex* species (follicle mites) and trombiculid mites (chiggers). Some animal and bird mites may bite man in the absence of their primary hosts and *Dermanyssus gallinae* (the chicken mite) is given as an example. Refer to Lane and Crosskey and Baker for keys to the families and genera of parasitic mites[9].

Preparation of material

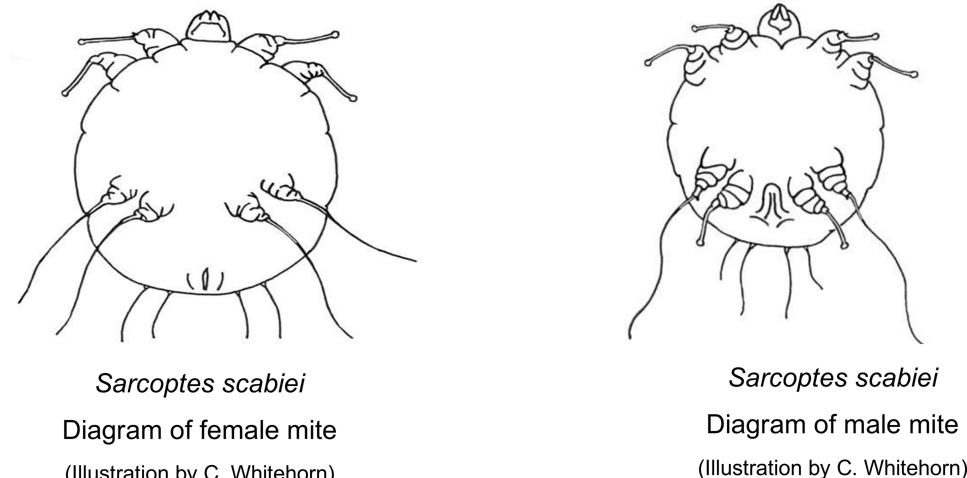
Mites should be killed and preserved in 70% ethanol or Oudeman’s solution prior to preparation (Oudeman’s solution prevents dehydration of specimens in long term storage. It consists of 87 parts 70% ethanol, 5 parts glycerine and 8 parts glacial acetic acid). Transfer soft-bodied or weakly sclerotized mites into a drop of Hoyer’s medium on a slide, add a coverslip and examine with care (Hoyer’s medium consists of 50mL distilled water, 30g crystalline gum Arabic, 200g chloral hydrate and 20mL glycerine). Sclerotized mites should be cleared prior to slide mounting by transferring to lactophenol for 4 to 72 hours till clear (Lactophenol consists of 50 parts lactic acid, 25 parts phenol crystals and 25 parts distilled water). When clear the mite should be rinsed three times in distilled water (10 minutes each rinse) and mounted in Hoyer’s medium as above. For a permanent preparation the slide should be baked at 50°C in an incubator for 4 days and the coverslip ringed with clear nail varnish. Label the slide with the identification, reference number and collection data[9].

Sarcoptes scabiei (the scabies mite)[3]

Cosmopolitan in distribution, *Sarcoptes scabiei* causes scabies in man and can affect people of any socio-economic class. A number of other species of *Sarcoptes* cause mange in pets and domestic animals but these mites are not viable on man. Scabies mites burrow through the upper layers of the skin feeding on the dermal tissues. Clinical symptoms result from sensitisation to the mites and to their faeces. Extensive and permanent burrows are only produced by the female mite and she is capable of living for up to two months on the human host. Scabies is transmitted from person to person only by close and prolonged physical contact.

Description

Scabies mites are very small; the males 0.2mm and the females 0.3 - 0.4mm. They have a striated cuticle bearing specialised scales and bristles. The legs are short and the forelegs bear specialised setae (pulvilli) to grip to the skin of the host.



Sarcoptes scabiei
Diagram of female mite
(Illustration by C. Whitehorn)

Sarcoptes scabiei
Diagram of male mite
(Illustration by C. Whitehorn)

Males can be differentiated from females by the presence of pulvilli on the hind pair of legs. These are used to hold on to the female during mating.

Lifecycle

The female mite lays eggs in her burrow as she tunnels through the skin. Eggs are oval and 0.1 - 0.15mm in length. They hatch in 3-8 days and a hexapod larva emerges. The larvae exit the burrow and migrate to the surface of the skin where they enter a hair follicle or burrow into the stratum corneum to construct a “moulting pouch”. The larva moults to an octapod nymph after 2-3 days. There are two nymphal stages prior to the adult stage. Mating occurs when the adult male penetrates the moulting pouch of a female. The impregnated female then extends her moulting pouch and begins her burrowing migration through the skin. The entire lifecycle takes 10-14 days and adult mites live for 4-5 weeks. The population of mites builds up over 2-4 months and a fully developed case of scabies will have an average population of 20 adult mites.

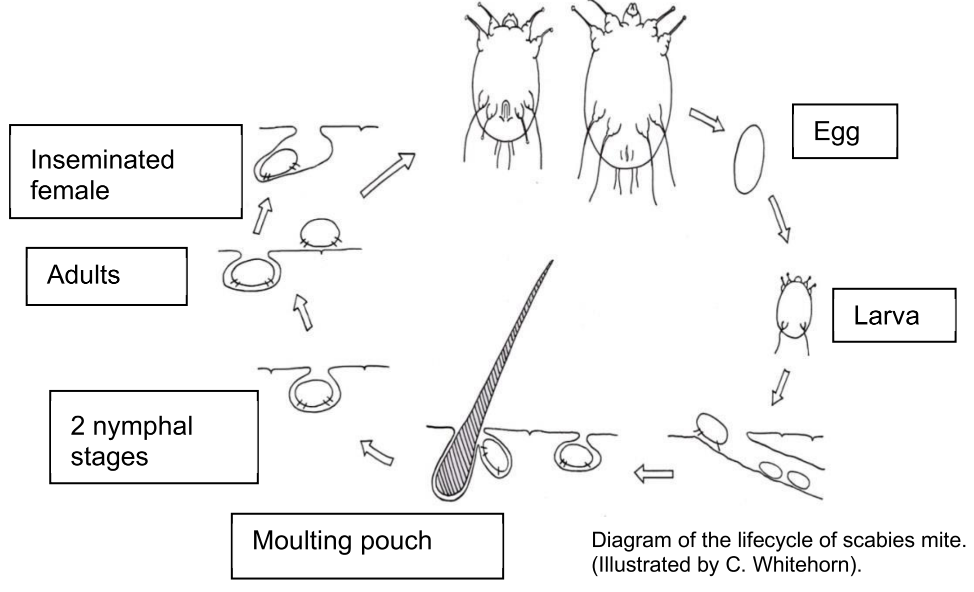
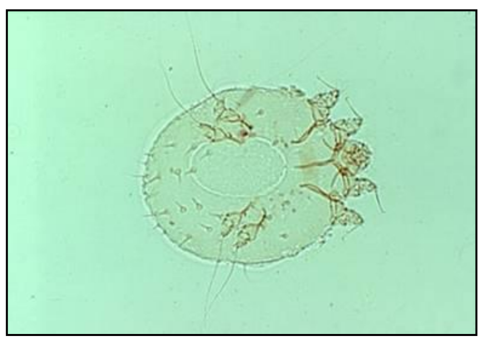


Diagram of the lifecycle of scabies mite.
(Illustrated by C. Whitehorn).

Pathology

When an individual is first infested with *Sarcoptes* there is seldom any evidence of symptoms for the first month (2-6 weeks). Clinical symptoms arise only when patients have become sensitized to the mites. Once sensitized, and in all subsequent infestations, symptoms may occur rapidly (in 1-4 days). Scabies presents as a generalised rash with raised, itchy papules occurring at the site of each mite burrow. Intense itching is one of the most commonly reported symptoms particularly at night and over most of the body. Scratching leads to broken skin and the formation of pustules. The majority of burrows are found between the fingers and the toes, and at the bends of the knees and the elbows, but the skin of the scrotum, penis, knees, buttocks and breasts may also be affected. The scabies “rash” is a symmetrical allergic skin reaction that may appear on the body from the underarms down to the calves and around the waist, but not on the upper back. The location of this “rash” does not correspond to the location of the mites. In patients with a suppressed immunity a condition called crusted ‘Norwegian’ scabies may occur. In this situation the patient does not react to the mites in the skin and the mite population increases unchecked. The condition results in a scaly crusted skin and is highly contagious. In children and the elderly scabies may be atypical in that the face and scalp may be affected and burrows are seldom found.



Sarcoptes scabiei

Photograph of female mite in slide preparation.
Note: the large egg located between the hind legs.

© LSHTM

Diagnosis

The diagnosis of scabies is based on the appearance and distribution of the rash and by the presence of burrows. Where possible the diagnosis should be confirmed by isolation of mites, eggs or faecal pellets from a skin scraping. Examine the surface of the skin for burrows using a magnifying glass. Pay particularly attention to the hands, the webs between the fingers and the folds of the wrist. Apply a drop of mineral oil to the skin where there is evidence of a burrow. With a sterile scalpel blade gently scrape the horny layer of the skin and collect the scrapings into a fresh drop of oil on a microscope slide. Examine the preparation under the x10 objective.

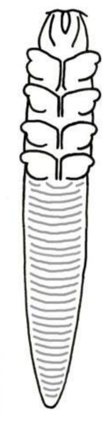
Demodex species (the follicle mites)

Follicle mites inhabit the skin of most adults especially women and normally produce no ill effects. They are host specific and two species are found on man: *Demodex folliculorum*, found in the hair follicles and *D. brevis*, found in the sebaceous glands. The entire lifecycle occurs in the follicles.

Description

Demodex species are extremely small mites (0.1- 0.4mm in length) and very atypical in that they have a worm-like appearance. The body is transversely striated and four pairs of stumpy legs are located anteriorly behind the mouthparts. The mites live head

down in the follicles and sebaceous glands and feed on subcutaneous tissues and exudates. Infestations occur mainly in the facial area, the eyelids and the nose.



Demodex species

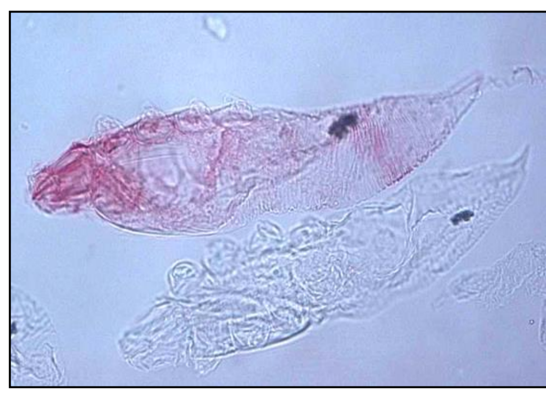
(Illustrated by C. Whitehorn).

Pathology

They can cause dermatitis in sensitised individuals and this may result in acne, rosacea, impetigo contagiosa or blepharitis.

Diagnosis

Express the contents of follicular pores around the naso-labial fold and smear on a microscope slide. Examine the preparation under the x40 objective.



Demodex species

Photograph of mite in slide preparation at x 340 magnification. Condenser iris must be set at 1 for maximum contrast.

© LSHTM

Trombiculid mites (chigger/scrub typhus mites)

The larvae of trombiculid mites are parasitic on vertebrates and are known as chiggers, harvest bugs and scrub-itch mites. They can cause dermatitis in man and are vectors of 'chigger-borne rickettsiosis' (scrub typhus) in South-East Asia. The nymph and adult stages are free-living predators.

Description

The larvae are oval, creamy white to reddish-orange in colour and are very small (0.15-0.3mm long). They have three pairs of legs which terminate in a pair of large claws. The palps and mouthparts are large and conspicuous and give the appearance of a head. The legs and the body are covered in fine feathered hairs. A dorsal shield (termed a scutum) can be seen on the anterior part of the body and from this a number of setae arise.

Lifecycle

Female mites lay eggs in leaf litter or damp soil. The eggs hatch after a week or so, but the larvae remain within the eggshell for a further 5-7 days before emerging. The hexapod larvae climb on to passing birds or mammals and then search for a suitable site to feed. Feeding lasts 2-10 days on man and then the engorged larvae drop to the ground, bury themselves in the soil and moult to become 8-legged protonymphs. The protonymph is a dormant stage and about one week later it moults to the deutonymph; a free living stage that feeds on soil animals. The deutonymphs are active for about 2

weeks and then undergo a further dormant period (the tritonymph) prior to the moult to the adult stage. The entire lifecycle typically takes 40-75 days.

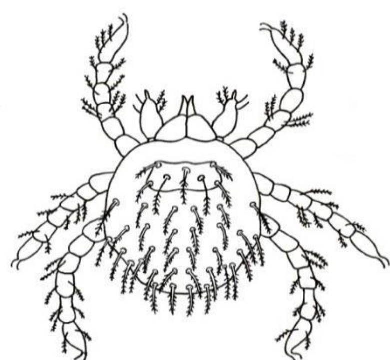
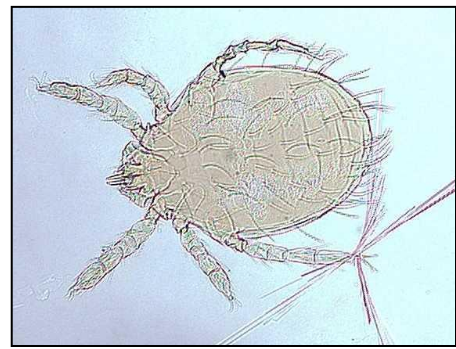


Diagram of a trombiculid mite (larva)
(Illustration by C. Whitehorn)



Leptotrombidium akamushi – larva
Photograph of a scrub typhus vector from Japan. © LSHTM

Pathology

The larvae attach to the skin of the host with their powerful mouthparts and inject saliva to the dermal tissues. The saliva digests these tissues and they are ingested by the larva. The repeated injection of saliva leads to the formation of a feeding tube that extends vertically in the host's skin. The bites cause an intense itchy dermatitis leading to pustules and wheals a few hours after exposure. This condition is known as 'harvest bug itch' or 'scrub itch'. Throughout Asia these mites are vectors of Rickettsia tsutsugamushi the causative agent of 'chigger-borne rickettsiosis', also known as scrub typhus. In cases where the mite is infected the bite is not painful, but an eschar is formed at the site of attachment. An eschar is a firm, black adherent scab 3-6mm in diameter and surrounded by a fine red margin. The symptoms of 'chigger-borne rickettsiosis' are typical of other forms of typhus and include fever and painful lymph nodes.

Diagnosis

Diagnosis is based on the location and presentation of lesions (stylostomes) on the skin surface. Mites particularly attach around the waist and genitals of man. Where mites are present on the host removal may be difficult, and great care should be taken when obtaining a sample for microscopy. Any material collected from the patient should be suspended in Berlese's fluid on a microscope slide and examined under the x10 objective for the presence of mites. Set the condenser iris at 1 for maximum contrast. Travel history may also be useful for diagnosis.

Dermanyssus gallinae (the chicken mite)

Commonly known as the red poultry mite or chicken mite *Dermanyssus gallinae* is a parasite of poultry and wild birds, and is cosmopolitan in distribution. In the absence of primary hosts (that is, when birds fledge and leave the nest) the mites will actively seek new hosts and will bite man.

Description

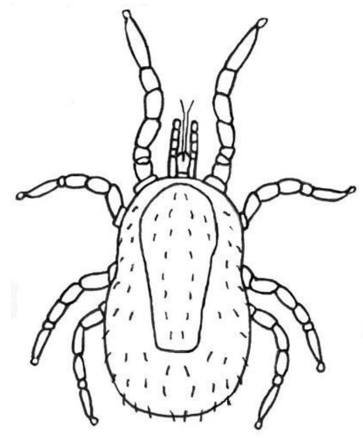
Unfed mites are roughly 0.7mm in length and grey in colour. They tend to feed at night and enlarge to become bright-red mites about 1.0mm in length. They have well developed legs and a large capitulum.

Dermanyssus gallinae

Photograph of anterior body.

© LSHTM

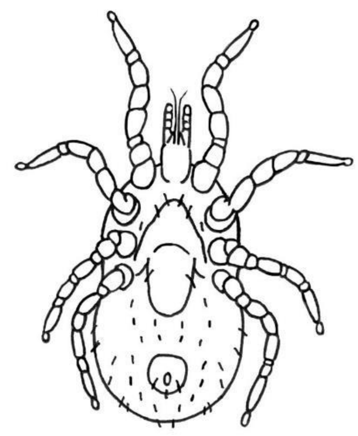
The chelicerae are long, whip-like and taper apically, but are very fragile and can be broken during specimen processing. The dorsal body surface has one shield. The anal shield on the ventral surface is cup shaped.



Dermanyssus gallinae

Diagram of dorsal surface of female mite.

(Illustration by C. Whitehorn)



Dermanyssus gallinae

Diagram of ventral surface of female mite.

(Illustration by C. Whitehorn)

Lifecycle

The eggs are deposited in the nesting material of the host and the cracks and crevices of the roost site. The lifecycle is extremely rapid and may be completed in one week given optimum conditions. Domestic infestations in houses, hospitals and tube stations can occur and are normally found to originate from abandoned nest sites.

Pathology

The bites of *D. gallinae* are painful and irritating.

Diagnosis

The mites are visible to the naked eye and may be collected from the host or from resting sites in the environment (cracks and crevices in furniture or walls). Chicken mites require a period of clearing in lactophenol prior to mounting for identification.

Ticks[1,2,10,11]

Ticks (subclass: Acari) are arachnids (class: Arachnida) and as such have a fused body that shows no division into head, thorax and abdomen. The body bears eight legs in the adult stages.

Ticks are obligate parasites of vertebrate hosts and have a high economic impact as ectoparasites of livestock and vectors of disease to animals and man[9]. Many will feed opportunistically on man and may be responsible for the transmission of zoonotic viral, rickettsial, bacterial and protozoal infections.

Refer to: Lane and Crosskey for keys to the families and genera of ticks and Hillyard (entire book) for keys to the ticks of North-West Europe[1,11].

Description

Ticks normally range in size from 1-30mm. The body is covered in a tough leathery integument. There is no division of the body into head, thorax and abdomen, but the capitulum (the structure that bears the mouthparts) is conspicuous and may be confused with a head. Ticks are generally larger than mites, but this is not a reliable character to distinguish the two groups. The presence of the toothed hypostome (median part of mouthparts) and the Haller's organ (on the tarsi of the first pair of legs) clearly distinguish ticks from mites.

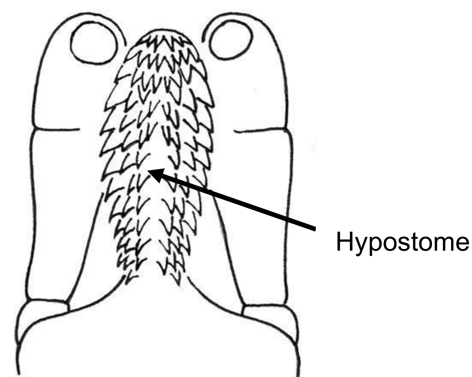


Diagram of ventral view of tick capitulum to show the basis capituli, the toothed hypostome (arrowed) and the palps.

(Illustration by C. Whitehorn)

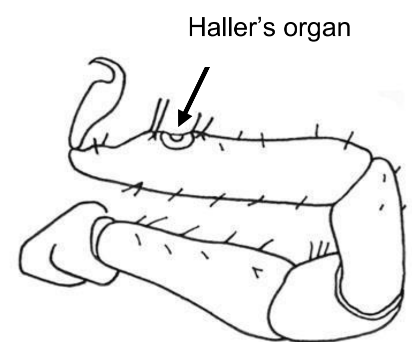


Diagram of tick foreleg to show Haller's organ (arrowed), a sensory organ.

(Illustration by C. Whitehorn)

Ticks demonstrate an incomplete lifecycle with the adults, nymphs and larvae occupying similar ecological niches. Adults and nymphs have eight legs, but larval ticks only have six legs. Larval stages could be confused with insects but for their lack of body division. There are two main families of ticks: the soft ticks (family: Argasidae) and the hard ticks (family: Ixodidae).

Soft ticks – *Argasidae*[10]

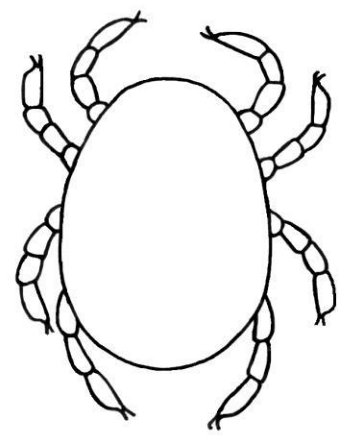
The soft ticks typically have an oval body shape and range in size from 4-15mm. They have a tough leathery integument that may be mammillated or granular in appearance. The mouthparts are located ventrally on the body surface and are therefore not visible from above. The lack of a scutal shield and the ventral location of the mouthparts help to distinguish soft ticks from hard ticks. There is little sexual dimorphism in soft ticks.

Lifecycle

The soft tick lifecycle is slightly more complex than that of the hard tick with a greater number of nymphal stages and longer life expectancy in adults. Eggs hatch to give a six-legged larva, the larva moults to give the eight-legged nymph (there can be 2 to 8 nymphal stages dependant on species). The nymph then moults to give the eight-legged adult. Soft ticks take more blood meals than hard ticks and over much shorter durations (15 – 120 minutes) so that they are seldom found attached to patients. Larvae take only one blood meal, but each nymphal stage and the adults will take two or more blood meals. Soft ticks are known as one-host ticks because they are normally associated with a single host species throughout their lifecycle. Females will lay a small batch of eggs after each blood meal and may live for over a decade. Soft ticks spend at least 99% of their time off the host. They are particularly resistant to desiccation and starvation and will enter a state of torpor to survive adverse conditions.

Dorsal view of a soft tick.

(Illustration by C. Whitehorn)



Medically important soft ticks

The soft ticks of medical importance are *Ornithodoros, Otobius* and *Argas.*

Ornithodoros

The genus *Ornithodoros* contains a number of species that are serious nuisance biters of man and seven species that are known to transmit tick-borne relapsing fever. Members of the genus can be recognised by their dark mammillated cuticle and absence of a distinct lateral line between the dorsal and ventral body surfaces. *Ornithodoros moubata* is probably the most important vector of tick-borne relapsing fever in tropical Africa but the disease is also found in South and Central America.

Ornithodoros moubata
Vector of relapsing fever.

© LSHTM

Otobius

Otobius megnini (the spinous ear tick) is primarily an ectoparasite of cattle and horses but will opportunistically attack man. The preferred site of attachment for the larva and nymph is the ear cavity and nymphs may remain attached for several months. The nymph ranges in size from 4 - 8mm and can be recognised by the presence of thick, dark spines over the body surface. The cuticle lacks a distinct lateral line between the dorsal and ventral body surfaces. *Otobius* is

restricted to the hot arid areas of the Americas, Africa and India. Adult *Otobius* species ticks are non-feeding.

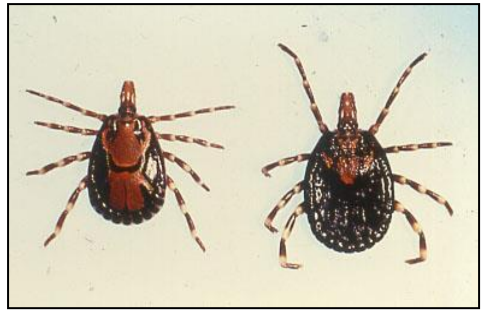
Argas

Members of the genus *Argas* are of worldwide distribution and are primarily associated with birds. These ticks will attack man opportunistically and have particularly painful bites. All life stages (larva, nymph, adult) may bite man but the larval Argas will remain attached to their host and continue to feed over a number of days. The body of the tick is slightly flattened with the dorsal and ventral body surfaces separated by a clear band of flattened rectangular cells.

Refer all soft ticks to a reference facility for identification.

Hard ticks – *Ixodidae*[10]

The hard ticks are recognised by the presence of a scutum, mouthparts that are visible from the dorsal surface, and marked sexual dimorphism in the adults. The scutum is an inflexible plate that lies on the dorsal surface of the body. It may bear a colourful pattern in some species. In males the scutum covers the entire dorsal surface but in females and immature stages it only covers the anterior part of the body (to allow full engorgement during feeding). Females are distinguished from nymphs by the presence of a genital aperture on the ventral surface and porose areas on the basis capituli (see photograph on page 39).



Amblyomma variegatum

Photograph of male and female.

© LSHTM

Lifecycle

The hard tick lifecycle is relatively simple with egg hatching to six-legged larva, larva moulting to eight-legged nymph and nymph moulting to eight-legged adult. Each stage requires a single feed before progressing to the next but it can take a number of years to complete the entire lifecycle. Hard ticks feed slowly and remain attached to the host for several days. Ixodids are often known as multi-host ticks because they are normally associated with two or three host species throughout their lifecycle (that is, larva – mouse, nymph – rabbit, adult – sheep). Females produce a single egg mass in their lifetime and die shortly afterwards. Hard ticks spend at least 90% of their time off the host resting and undergoing development. They are very sensitive to desiccation and extremes of temperature.

Medically important hard ticks

Members of the genus ***Ixodes*** are known to be vectors of Lyme disease in the UK[11]. Two of the most commonly referred species are ***Ixodes ricinus*** and ***Ixodes hexagonus.*** The genus can be identified by examining the anal groove on the ventral surface of the tick[11].

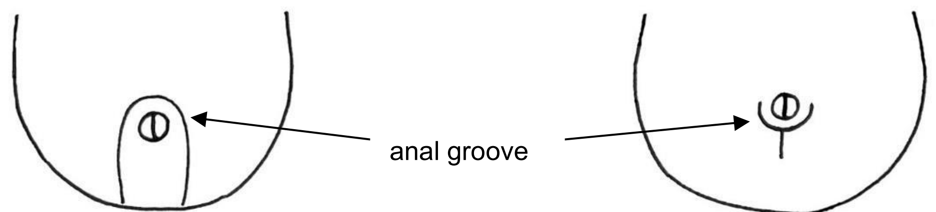


Diagram of the anal groove of ticks belonging to the genus *Ixodes*.

(Illustration by C. Whitehorn).

Diagram of the anal groove of all other hard tick genera.

(Illustration by C. Whitehorn)

For *Ixodes* the anal groove circles the anus anteriorly. For all other hard tick genera the anal groove circles the anus posteriorly.

If the patient has been bitten by a British tick of another genus there is little risk of disease transmission.

Refer any non-*Ixodes* species and all ticks acquired overseas to the appropriate reference facility for identification.

Lyme disease[12]

Lyme disease is a bacterial infection caused by the spirochaete *Borrelia burgdorferi*. It is a zoonotic infection associated with woodland and heath land habitats. The main reservoir hosts are rodents (wood mice and voles) and the deer population. In unfed ticks the spirochaetes are restricted to the midgut and only migrate to the salivary glands once feeding is initiated. Therefore swift removal of ticks (within 24-48 hours of attachment) is an important factor in reducing the risk of disease transmission.

Tick paralysis

Tick paralysis is caused by the inoculation of a neurotoxin (within the saliva) into the host as a female tick feeds. A number of hard tick species are known to cause this condition, but none are native to the UK. Five to seven days after attachment the host may develop fatigue, numbness and muscle pain. This may rapidly progress toward partial paralysis, convulsions and respiratory failure unless the attached tick is located and carefully removed. Symptoms will persist as long as any part of the tick's mouthparts remain in the host.

Removal of ticks

Forcible removal of ticks often results in damaged mouthparts and the risk that material is left embedded in the skin of the patient. Mouthparts also provide important characters for the identification of ticks so using the correct removal technique is vital. Take a pair of fine forceps and gently (but firmly) grasp the mouthparts of the tick as close to the patient's skin as possible. Rotate the mouthparts slightly whilst pulling the tick away from the skin. A firm but gentle action should result in the removal of the tick without harm to either party. Transfer the tick to a sterile tube and refer for identification. Clean and disinfect the wound site and sterilize the forceps. Monitor the wound site over the next few days for any developing erythema.

Preparation of material

Ticks should be killed in hot water (85°C) and preserved in 70% ethanol. Adult ticks should be examined under a dissecting microscope, but temporarily removed from the

ethanol and examined dry. Body features are more clearly visible as the ethanol evaporates. Nymphs and larvae should be slide mounted in Hoyer's medium and examined under a compound microscope. Hoyer's medium consists of 50mL distilled water, 30g crystalline gum Arabic, 200g chloral hydrate and 20mL glycerine[9].

The life stages

Keys for the identification of ticks are written for each of the life stages; larva, nymph, male and female. External characters may vary considerably among the life stages even within the same species. It is therefore crucial that the life stage is identified correctly before attempting to key out the specimen any further.

Life stages of soft ticks

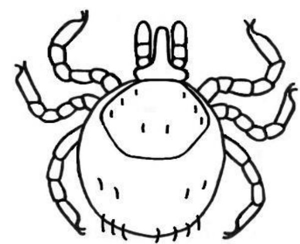
As previously stated there are only minor morphological differences between the life stages of the soft ticks. Larvae are distinguished by having only six legs, the nymphs by having eight legs and lacking a genital aperture, and the adults by having eight legs and possessing a genital aperture. In the adults the genital aperture is located on the ventral surface, below the mouthparts, along the midline between coxae 1 and 2. In females it appears as a horizontal slit and is often strongly marked. In males the genital aperture is oval and less clearly defined.

Life stages of hard ticks

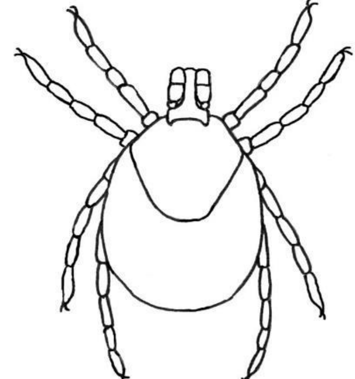
Although there are greater morphological variations among the life stages of the hard ticks it is still necessary to examine specimens with care. This is particularly important when differentiating between the nymph and the female hard tick. The larval stage is distinguished by having only six legs and the nymph by having eight legs and lacking a genital aperture. Adults have eight legs and possess a genital aperture. The genital aperture is located on the ventral surface of the body, over the midline, between coxae 3 and 4. The sexes are differentiated by the size of the scutal shield; the scutum completely covers the dorsal surface of the body in male ticks but only covers the anterior part of the body in female hard ticks. Confusion often arises between the nymph and the female stage because they both have a reduced scutum and eight legs, so in addition to the genital aperture another character to look for are the porose areas on the dorsal basis capitulum. These are absent in nymphs but present in the females.

Diagrams of the life stages of hard ticks. Dorsal view.

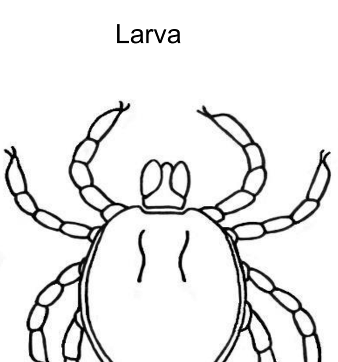
(Illustrated by C. Whitehorn)



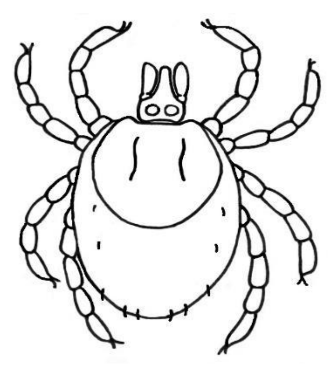
Larva



Nymph



Male



Female

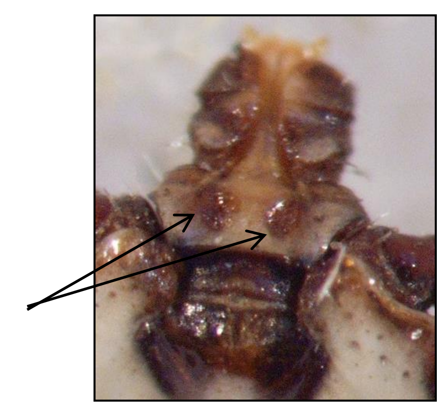
Porose areas

Two depressions located on the dorsal surface of the basis from which the females produce secretions to lubricate and protect the egg mass. The porose areas can merge to form a single depression in some species of hard ticks.

Rhipicephalus pulchellus.

Photograph of female to illustrate the porose areas on the basis capitulum.

© LSHTM



Of all the tick specimens that are referred for identification the hard tick *Ixodes ricinus* is the most common. On the following pages are descriptions of all the life stages of *Ix. ricinus* and of *Ix. hexagonus* (another commonly referred species) for your information.

Ixodes ricinus (common sheep tick, castor bean tick or wood tick)[10]

This tick is distributed throughout North-West Europe and most of the western Palaearctic, it is most abundant in the sheep pastures of the British Isles.

Description

A brief description is given for all life stages but the nymph and the adult female are most commonly sent for identification. Please refer to P. Hillyard (pg 74-76) for illustration of the structures listed below[11]. The description of the larva is taken from Arthur[10].

Larva

Length of unfed larva approximately 1mm. The larval stage has only 3 pairs of legs and the scutum only partially covers dorsal surface of body. The palps (segments 2 and 3) are longer than the width of the basis capituli. The denticles on the hypostome are arranged in two or three rows of 3/3 distally, then six or seven of 2/2. The auriculae (structures on the ventral surface of the basis) appear as distinct projections. The scutum is wider than long and roughly hexagonal in shape. Coxae 1 to 3 have distinct external spurs and coxa 1 has a small internal spur.

Nymph[11]

Length of unfed nymph 1.3–1.5mm. The nymph has four pairs of legs, the scutum only partially covers dorsal surface of body and there is no genital pore on the ventral surface. The palps (segments 2 and 3) are longer than the width of the basis capituli. The scutum is almost circular in shape, the auriculae (structures on the ventral surface of the basis) resemble divergent triangles, and coxa 1 (the first segment of the first leg) has an internal spur (pointed outgrowth on coxa) longer than the external spur.

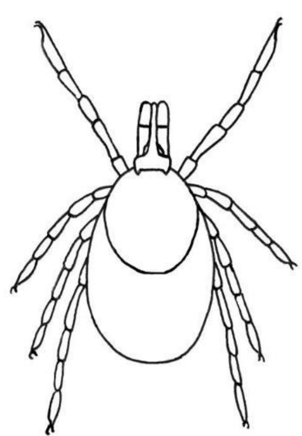


Diagram of dorsal surface of *Ixodes ricinus* nymph.

(Illustration by C. Whitehorn)

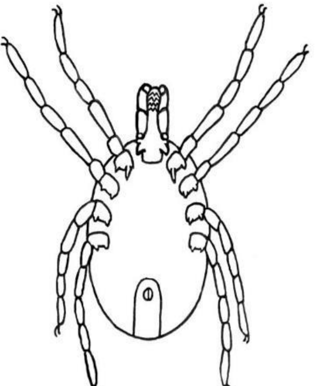


Diagram of ventral surface of *Ixodes ricinus* nymph.

(Illustration by C. Whitehorn).

Adult Male[11]

Length of male 2.4–2.8mm. The scutum covers dorsal surface of the body. The palps are short and broad. The hypostome has prominent teeth. The internal spur of coxa 1 is three times longer than external spur. Tarsus 1 (in profile) tapers gradually.

Adult Female[11]

Length of unfed female 3.0–3.6mm. The scutum partially covers dorsal surface of the body, a genital pore is present on the ventral surface and two porose areas are present on the dorsal basis capituli. The palps (segments 2 and 3) are longer than the width of the basis. The scutum is a little longer than wide and broadly rounded posteriorly. Auriculae are absent. Coxa 1 has a long internal spur. Tarsus 1 (in profile) tapers gradually. The genital aperture is located between coxae 4.

Ventral view of *Ixodes ricinus* female to show anal and genital grooves.

© LSHTM

Dorsal surface of an *Ixodes ricinus* female (unfed)

© LSHTM

Ixodes hexagonus (hedgehog tick)[10]

Distributed widely throughout Western Europe this tick is a parasite of hedgehogs, foxes, badgers and dogs. *I. hexagonus* frequently bites man.

Description

A brief description is given for all life stages but the nymph and the adult female are most commonly sent for identification. Please refer to P. Hillyard for illustrations of the structures listed below[11]. The description of the tick larva is taken from Arthur[10].

Larva

Length of unfed larva approximately 1mm. The larval stage has only 3 pairs of legs and a scutum that only partially covers the dorsal surface of the body. The palps (segments 2 and 3) are approximately equal in length to the width of the basis capituli. The denticles on the hypostome are arranged in two or three rows of 3/3 distally, and then about four rows of 2/2. The auriculae are represented as thickened ridges. The scutum is usually wider than long and heart-shaped. Coxa 1 has a broad internal spur but no other spurs are present.

Nymph[11]

Length of unfed nymph 1.2–1.4mm. Nymph has four pairs of legs, the scutum only partially covers dorsal surface of body and there is no genital pore on the ventral surface. The palps (segments 2 and 3) are slightly shorter than the width of the basis capituli. The scutum is longer than wide and hexagonal in shape. No auriculae are present. The internal spur on coxa 1 is short, external spurs on coxae 1-4 are reduced or absent.

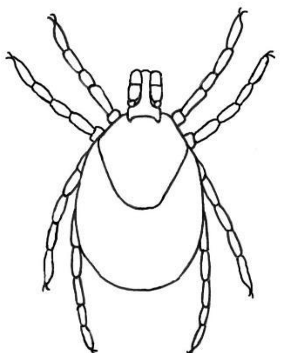


Diagram of dorsal surface of *Ixodes hexagonus* nymph

(Illustration by C. Whitehorn)

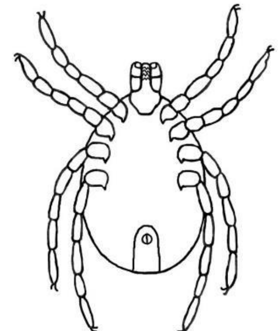


Diagram of ventral surface of *Ixodes hexagonus* nymph

(Illustration by C. Whitehorn)

Adult male[11]

Length of male 3.5–3.8mm. The scutum covers dorsal surface of body. The body is broadly oval in shape. The palps are short and broad and the hypostome is almost toothless. The internal spur on coxa 1 is long. Tarsus 1 (in profile) is clearly stepped near apex.

Adult female[11]

Length of unfed female 3.5–4.0mm. The scutum partially covers the dorsal surface of the body, the genital pore is present on the ventral surface and two porose areas are present on the dorsal basis capituli. The palps (segments 2 and 3) are slightly shorter than the width of the basis. The scutum is characteristically heart or hexagonal in shape. The auriculae are vestigial. Coxa 1 has a long internal spur. Tarsus 1 (in profile) is clearly stepped near apex. The genital aperture is located between coxae 3.

Dorsal surface of an *Ixodes hexagonus* female (part fed).

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Maggots[2]

Myiasis maggots[2,3]

Myiasis is caused when fly maggots (dipterous larvae) invade living vertebrate animals to feed on living tissue, necrotic tissue, body fluids or the ingested food of the host[13]. Myiasis causing flies are either obligate or facultative parasites in their larval stages: the former must develop on or in a living host, the latter can also develop on decaying organic matter. The facultative parasites may be primary, secondary or tertiary invaders of vertebrate animals depending on their ability to attack hosts. Primary invaders initiate myiasis, but secondary and tertiary invaders only attack the host once myiasis has been initiated by other species. However it should be noted that primary

invaders (such as the greenbottle *Lucilia sericata*) always require a predisposing condition such as a wound or poor hygiene to stimulate oviposition. They will not initiate myiasis on clean, healthy animals.

A condition known as pseudomyiasis may occur when the larvae or eggs of certain species are accidentally ingested and pass living through the gut of the host.

Description

The myiasis maggots do not have a uniform appearance because they are drawn from a large number of dipteran families. However, those of principal medical importance exhibit two main body shapes; the classic wedge-shaped maggot and the more rounded grub-like maggot. The body is divided into 12 segments, the first being the head, the next three the thorax and the last eight the abdomen. However, there is little differentiation between the segments. The first segment contains the cephalopharyngeal skeleton (the mouth parts) and this has important taxonomic characters for identification. The second segment bears a pair of anterior spiracles which link via two tracheal trunks to the posterior spiracles on the 12th (terminal) body segment. The spiracles and tracheae are the respiratory apparatus of the maggot and also provide useful characters for identification. Myiasis maggots do not have legs, but some species have body swellings, spines and processes that aid in locomotion and prevent dislodgement from the host.

Medically important myiasis maggots

The genera that are principally referred for identification in the UK are *Lucilia* (greenbottles), *Calliphora* (bluebottles), *Oestrus* (sheep nasal bot-fly) and *Sarcophaga* (flesh flies)[14]. *Cordylobia* (Tumbu fly and Lund's fly) and *Dermatobia* (human bot-fly) are both tropical genera that are occasionally seen in overseas travellers returning to the UK. There are a number of other genera that are serious agents of myiasis and many more that are of minor medical importance. This UK SMI will examine only those genera that are commonly referred for identification. All the descriptions included are for third instar larvae, except for *Oestrus ovis* where the first instar larva is described. Refer to Lane and Crosskey for keys to the families and genera of myiasis maggots[1].

Preparation of material

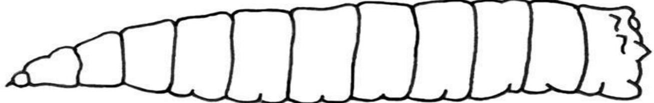
Myiasis maggots should be killed by immersion in hot or boiling water (90-100°C) for 15-30 seconds and then stored in 80% ethanol prior to preparation. Take the maggot and slice off the last segment that bears the posterior spiracles. Place the segment and the body into a test tube containing 5% Potassium hydroxide (KOH), transfer to a water bath and slowly heat to boiling point. Remove from heat and allow the contents to cool for 10 minutes. Wash the body and posterior segment well in water (two changes of about 5 minutes each). Transfer the maggot body to a glass slide and begin to squeeze the body contents out from the cuticle. It may be necessary to repeat the KOH boiling stage two or three times to extract all the body tissues. Dehydrate the body cuticle and the posterior segment through increasing strengths of ethanol (70%, 90% and absolute) for five minutes each. Repeat in fresh absolute alcohol for five minutes before transferring to Cellosolve (2-ethoxyethanol) for a final five minutes. Mount the specimen directly in euparal in the centre of a microscope slide. The body cuticle should be placed laterally on the upper half of the slide and the posterior segment below (outer cuticle uppermost). Add a coverslip and carefully examine the specimen. The slide specimen should be placed into an oven for 4-6 weeks at 55°C to

give a permanent preparation. Label the slide with the identification, reference number and collection data.

Lucilia – greenbottles

Greenbottles are cosmopolitan in distribution. Female flies lay their eggs in neglected wounds and on soiled fabrics. The larvae feed directly on the tissues or on necrotic slough. Females will also deposit eggs on raw and cooked meats or fish and if this is ingested intestinal myiasis may occur[7].

Description: Maggots of the genus *Lucilia* have the classic "maggot" form with a narrow anterior segment bearing the mouth-hooks broadening out to a truncate posterior segment.

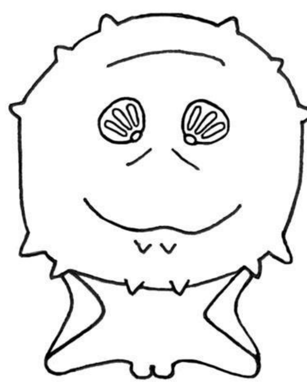


Lucilia species.

Diagram of third instar larva.

(Illustration by C. Whitehorn)

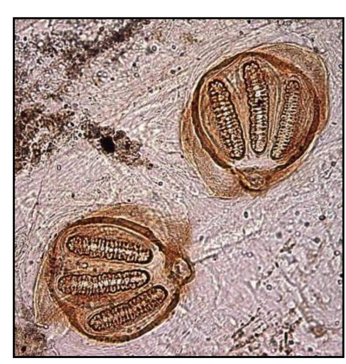
A mature larva is about 14mm long and white to cream in colouration. The posterior spiracles are located on the face of the terminal segment and each consists of three straight slits surrounded by a closed peritremal ring with a distinct button.



Lucilia species.

Posterior view of terminal segment of third instar larva.

(Illustration by C. Whitehorn)



Lucilia species.

Photograph of posterior spiracles.

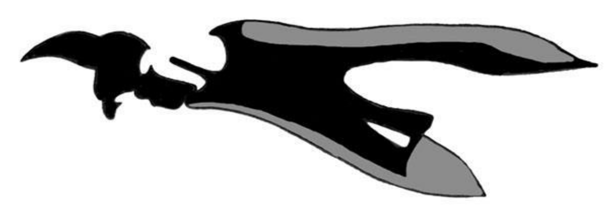
© LSHTM

There is no accessory oral sclerite (a small additional sclerite) present between the mouth-hooks.

Lucilia species.

Photograph of anterior segments to show skeleton, cephalopharyngeal and anterior spiracles.

© LSHTM



Lucilia species.

Diagram of cephalopharyngeal skeleton. **Note:** Lack of accessory oral sclerite between mouth-hooks.

(Illustration by C. Whitehorn)

Calliphora – bluebottles

Bluebottles are cosmopolitan in distribution. Female flies typically lay their eggs on decaying organic matter and normally attack man only as secondary invaders. Females will also deposit eggs on fresh meat and if that is ingested an intestinal myiasis may occur.

Description

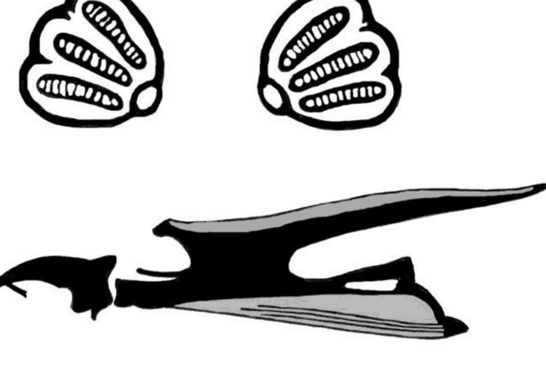
Maggots of the genus *Calliphora* are slightly larger than those of *Lucilia* but have an identical body shape and colour. A mature larva is about 17mm long. The posterior spiracles are located on the face of the terminal segment and each consists of three straight slits surrounded by a closed peritremal ring with a distinct button. *Calliphora* species are distinguished from *Lucilia* species by the presence of an accessory oral sclerite between the mouth hooks.

Posterior spiracles of *Calliphora* species. (Illustration by C. Whitehorn)

Calliphora species. Photograph of cephalopharyngeal skeleton.

Note: presence of accessory oral sclerite between mouth-hooks.

© LSHTM



Calliphora species. Diagram of cephalopharyngeal skeleton.

Note: accessory oral sclerite between mouth-hooks.

(Illustration by C. Whitehorn)

Cordylobia anthropophaga – The Tumbu fly

The Tumbu fly occurs throughout sub-Saharan Africa. Female flies lay their eggs on damp sandy soil or on clothing hanging in the shade to dry (sites soiled by sweat, faeces or urine are particularly favoured). The larvae emerge after 2 days and, when triggered by the presence of a host, burrow into the subcutaneous tissues. The larvae remain within the dermis and each forms a distinct boil-like swelling. There are 3 larval instars and these take 8 -12 days to complete. The mature larva emerges from the "boil" and falls to the ground to pupate.

Description

Maggots of the genus *Cordylobia* have a grub-like appearance being fleshy and rounded at both ends. A mature *C. anthropophaga* larva is approximately 12mm long and 5mm wide. The third to eleventh segments of the body are densely covered with small spines. The posterior spiracles are located on the face of the terminal segment. Each spiracle consists of three moderately sinuous slits that are not surrounded by a peritremal ring but do have a faint button.

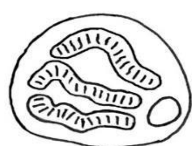
Cordylobia anthropophaga

Photograph of third instar larva with scale in millimetres.

© LSHTM.

Spines on the cuticle of *Cordylobia.*

© LSHTM



Cordylobia anthropophaga

Diagram of posterior spiracles of third instar larva.

(Illustration by C. Whitehorn).

Cordylobia anthropophaga
Photograph of posterior spiracles.

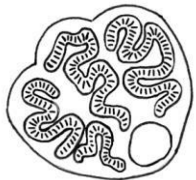
© LSHTM

Cordylobia rodhaini – Lund's fly

Lund's fly occurs in the rainforest areas of tropical Africa and is very similar in appearance and lifecycle to the Tumbu fly.

Description

The maggot has a grub-like appearance and a mature *C. rodhaini* larva is 17-33mm long and 8mm wide. The body is covered in numerous large spines. The posterior spiracles are located on the face of the terminal segment.



Cordylobia rodhaini

Diagram of posterior spiracles of third instar larva.

(Illustration by C. Whitehorn)

Cordylobia rodhaini

Photograph of posterior spiracles of third instar larva. © LSHTM

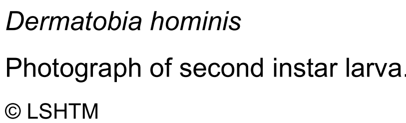
Each spiracle consists of three long serpentine slits that are not surrounded by a peritremal ring but do have a faint button.

Dermatobia hominis – the human bot-fly

The human bot-fly occurs throughout Central and South America. Female flies deposit batches of eggs on mosquitoes and other biting flies, or even ticks. These "couriers" then locate a host and as they feed, the bot-fly eggs (stimulated by the host's temperature and odours) hatch. The larvae burrow into the skin of the host and each forms a distinct boil-like swelling. There are three larval instars and these take 6-12 weeks to complete. The mature larvae then emerge from the "boil" and fall to the ground to pupate. The "boils" are painful for short periods during larval feeding, and are less tender during developmental periods. The discharge of fluids (larval faeces and the host's body fluids) from the wound may provide an attractive oviposition site for other myiasis flies.

Description

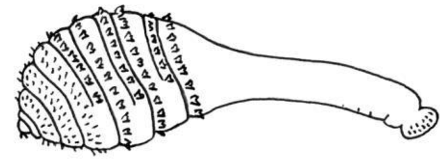
The maggot has a characteristic "pear-shaped" appearance with a swollen anterior end and a narrow posterior end. The body shape makes it particularly difficult to dislodge when embedded in the host. A mature larva is about 20mm long and 8mm at the widest part. The anterior and central segments bear numerous spines. The posterior segments have no spines but small denticles are present on the terminal segment. The posterior spiracles are recessed into a cavity on the terminal segment and consist of three straight slits with no peritremal ring and no button.



Dermatobia hominis

Photograph of second instar larva.

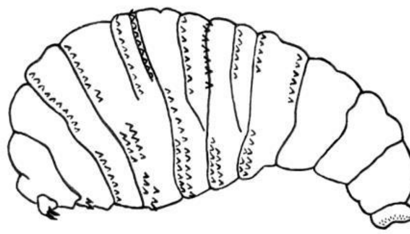
© LSHTM



Dermatobia hominis

Diagram of second instar larva.

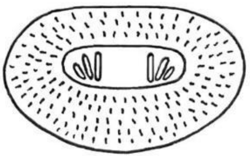
(Illustration by C. Whitehorn)



Dermatobia hominis

Diagram of third instar larva.

(Illustration by C. Whitehorn)



Dermatobia hominis

Posterior view of terminal segment to show spiracles.

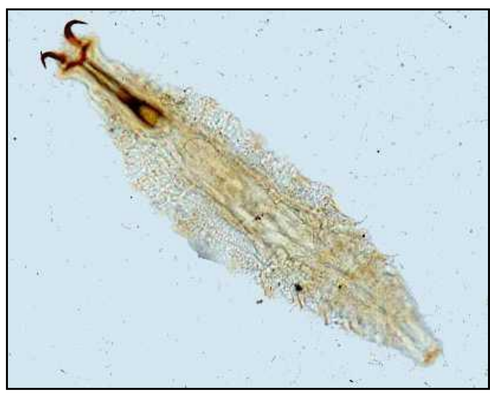
(Illustration by C. Whitehorn)

Oestrus ovis – the sheep nasal bot-fly

The sheep nasal bot-fly is worldwide in distribution and particularly common where sheep and goats are reared. The female fly deposits live first instar larvae directly on the host with the nasal passages being the primary larviposition site followed by the eyes, mouth and ears. People, especially those working with livestock, may occasionally be attacked. The most common history is a patient who reports being struck in the eye by a small object and who goes on to develop a painful inflammation of the eye in the following few hours. An acute catarrhal conjunctivitis may be diagnosed. In man *Oestrus* is generally non-invasive and the first instar larva is unable to develop further, however it may remain viable for ten days causing discomfort to the patient throughout that period. Larvae need to be removed from the conjunctival sac by an ophthalmologist.

Description

The first instar larvae are very small (1.0mm long), elongate, oval and transparent but the large mouth hooks are particularly distinct and are the diagnostic feature.



Oestrus ovis

Photograph of first instar larva.

© LSHTM

Oestrus ovis

Diagram of cephalopharyngeal skeleton of first instar larva.

(Illustration by C. Whitehorn)

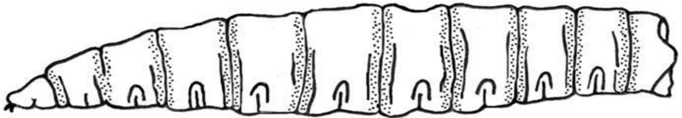
The strong buccal hooks are recurved and horn-like and may be withdrawn into the body. Small spines are present on each body segment and the last segment bears two prominences each possessing a number of hooklets.

Sarcophaga – flesh flies

The flesh flies are of cosmopolitan distribution. Female flies lay first instar larvae directly on decaying organic matter or faeces and normally attack man only as secondary invaders. The exception is *Wohlfahrtia magnifica* which is an obligate, primary invader. *Sarcophaga* species have been recorded infesting the bedsores of elderly patients. Female flies will also deposit larvae on foodstuffs, and if these are ingested an intestinal myiasis may occur.

Description

Maggots of the genus *Sarcophaga* have the classic "maggot" form with a narrow anterior segment bearing the mouth-hooks broadening out to a truncate posterior segment. A mature larva is about 16-22mm long.



Sarcophaga species

Diagram of third instar larva.

(Illustration by C. Whitehorn)

Sarcophaga species

Posterior view of terminal segment.

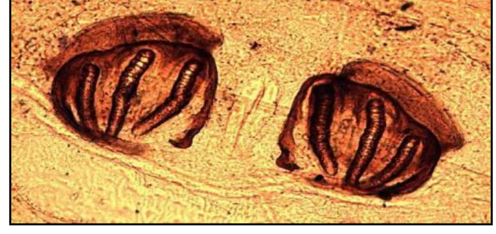
(Illustration by C. Whitehorn)

The posterior spiracles are recessed into a deep cavity on the terminal segment and can be concealed when the cavity is closed. The spiracles themselves consist of three straight slits surrounded by an open peritremal ring with no button.

Sarcophaga species.

Diagram of the posterior spiracles of third instar larva.

(Illustration by C. Whitehorn)



Sarcophaga species.

Photograph of posterior spiracles.

© LSHTM

Leeches

Description

Leeches (Euhirudinea) are hermaphroditic annelids, which feed by sucking blood and tissue fluids from their hosts[15-17]. Aquatic and terrestrial forms exist.

Aquatic leeches have a worldwide distribution. Terrestrial species are found in South-East Asia, the Pacific islands, the Indian subcontinent, and South America[18]. Terrestrial leeches inhabiting the tropical rain forests, and attaching to the skin of man and animals, are a great nuisance. However, individual bites of such leeches are generally medically trivial, and are not usually associated with infection, or with transmission of disease, but evidence for persistence of various pathogens within the bodies of leeches has been reported, including blood-borne[19].

Iatrogenic wound infection with *Aeromonas* species is described, especially in the context of medicinal use of leeches (principally *Hirudo medicinalis*, but other species have been used[20]). These leeches have a symbiotic relationship with bacteria of the genus *Aeromonas*, which inhabit their intestine often in pure culture, but other potentially pathogenic bacteria may also be present[20-23].

H. medicinalis may be applied to reduce tissue engorgement consequent upon plastic surgical or vascular reconstructive manoeuvres. The engorged tissue may be particularly susceptible to infection by *Aeromonas* species.

Once a leech attached to the skin has finished its meal, it will generally detach spontaneously. Bleeding may continue for many minutes or hours, unless staunched by an appropriate dressing (during feeding, an anticoagulant – hirudin - is injected by the leech).

An attached leech should be allowed to feed for a period so that there is a flow of blood out of the wound. This may help to carry away any microbes that may have been introduced to the lesion.

Medicinal leeches must only be subject to single-patient use.

The first indication of a wild leech bite is often the discovery of blood in clothing, the leeches having already fed, engorged and detached. There is controversy on this point, but it is thought desirable by some to cause an attached terrestrial leech to detach, by application to the organism of heat from a burning cigarette or match, or application of a strong solution of salt, or alcohol, rather than simply pulling it off directly, which may leave mouthparts still embedded in the wound[18].

Certain South American leeches do not possess jaws, and feed by insertion of a proboscis deep beneath a host's tissue. Violent removal of these leeches could leave a long tube of leech tissue in the host.

Some of the aquatic (nasopharyngeal) species are capable of causing very serious disease in humans and animals, and sometimes prove fatal. Such leeches are to be found in fresh waters in parts of the Middle East and Africa, the Indian sub-continent, and China.

These leeches are small and thread-like when first hatched, and enter the bodies of drinking animals or humans, or bathers in surface waters, attaching to the mucous tissue in the nasopharyngeal and buccal cavities, occasionally the oesophagus, or the tracheobronchial tree. Leeches may remain attached for several weeks, before emerging fully grown. Growth within the host is dramatic, and one species, *Dinobdella ferox,* may attain a length of 250mm.

Aquatic (nasopharyngeal) leeches may also enter the urethra or vagina in swimmers and bathers, or attach to the conjunctiva.

Obstruction, epistaxis, haemoptysis, haematemesis and severe anaemia may result from attachment of aquatic (nasopharyngeal) leeches. Leeches may be surgically

removed – application of cocaine or hypertonic saline to the leech may facilitate detachment of the organism[18,24]. Tracheostomy may be necessary to gain access, or to relieve life-threatening respiratory obstruction.

Protection

Complete protection against leeches is almost impossible. In tropical climates chemical repellents only work for a limited period due to sweating. Terrestrial leeches are extremely slender, very agile, and are capable of squeezing through boot eyelets, and all but the most tightly woven cloth. However, travellers should ideally wear thick trousers treated with a repellent such as diethyl toluamide, tucked into the tops of stout boots.

Where nasopharyngeal leeches occur, drinking water should be strained through a tight mesh, or preferably boiled.

Swimming or bathing in untreated surface waters overseas is always most unwise, for a variety of reasons.

Use of antibiotic prophylaxis may be indicated if medicinal leeches are to be applied to engorged tissues as a part of post-operative surgical care[20]. Ciprofloxacin and extended-spectrum beta-lactam agents may be considered for this indication, depending on the antibiotic sensitivity of the *Aeromonas* species that the leech is carrying[23,25].

Preservation of specimens

To ensure specimens are suitable for taxonomic anatomy, live leeches should be narcotised in 15% alcohol. When fully narcotised the leeches should be extended flat for fixing in 70% alcohol or 5% formalin. Specimens dropped live to formalin or concentrated alcohol contract violently, harden and are useless for identification.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[26,27]

UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must

allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

1 Safety considerations[26-41]

1.1 Specimen collection, transport and storage[26-31]

Personnel should wear gloves, a laboratory coat and work in a well-lit, clear laboratory space.

Containment Level 2 is required for routine work.

In some cases ectoparasites may be engorged with human blood or body tissue, this may pose a risk to the person handling them if infected with a Hazard Group 3 organism. If a Hazard Group 3 organism is suspected, a microbiological safety cabinet at Containment Level 3 is required.

Any specimen contaminated by the body fluids or blood of the patient should be immersed in 10% formol water at a ratio of 1:3 (specimen: formol water) for a minimum of 30 minutes prior to being processed.

Specimens should be handled with forceps or a fine brush. When processing material the use of sterile scissors is recommended in preference to a scalpel blade.

Live specimens should be killed prior to identification unless required live for the isolation of pathogens. Live specimens should be handled with extreme care and chilled in a refrigerator if there is any risk of escape during processing. Live specimens are killed with hot water (85°C) (ticks, lice, fleas, bedbugs, myiasis maggots), or with ethyl acetate (beetles and adult flies) or killed by ethyl acetate vapour. Please refer to the appropriate section for further details.

Some specimens may require a period of clearing in hot 5% potassium hydroxide solution prior to identification, in which case appropriate protective equipment must be worn.

Alcohol and organic solvents such as Euparal should not be used near naked flames and should be used in an area with good ventilation. Solvents should not be used in microbiological safety cabinets as they present an ignition risk and may promote damage to the HEPA filters.

The containers of specimens preserved in alcohol should be tightly sealed and stored in lockable metal cabinets.

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[26-41]

Specimens should be handled with care using forceps or a fine brush to avoid damage to taxonomic features required for identification.

Specimens that have been stored in formalin or washed in formol water may toughen and be more difficult to process. It is recommended that they are transferred in to 70% ethanol as soon as possible.

Some flea specimens may require a period of clearing prior to examination. The immersion of specimens in 5% KOH for several days is normally adequate.

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[34].

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Ectoparasitic arthropods, leeches

2.2 Optimal time and method of collection[42]

Specimens should be handled with care to avoid damage to taxonomic features required for identification. Specimens should be collected directly from the patient whenever possible or from the environment in which the patient lives. Specimens should be collected into a CE marked leak proof container in a sealed plastic bag[26].

Ideally specimens should be killed before postage. All soft-bodied specimens (lice, fleas, bedbugs, ticks and fly larvae) should be killed by immersion in hot water, transferred to and transported in 70% ethanol. All hard bodied specimens (including beetles and adult flies) should be killed by exposure to ethyl acetate vapour and transported dry. Mites may be killed and transported in 70% ethanol. Refer to the appropriate section for further details. A short patient history and details of any foreign travel should be included.

Inner packaging containing the specimen should be examined prior to opening to ascertain if the insect/arachnid is still living. Living specimens should be killed with hot water or ethyl acetate vapour prior to examination. Where there is a risk that live specimens may escape when the container is opened, they should be chilled in a refrigerator before processing.

To ensure specimens are suitable for taxonomic anatomy live leeches should be narcotised in 15% ethanol. When fully narcotised the leeches should be extended flat for fixing in 70% ethanol or 5% formalin. Specimens dropped live to formalin or concentrated ethanol contract violently, harden and are useless for identification.

For safety considerations refer to Section 1.1.

Collect specimens before starting treatment where possible[42].

Collect specimens into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens[42]

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

3 Specimen transport, storage and retention[26,27]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[42].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[42].

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'[43].

4 Specimen processing/procedure[26,27]

4.1 Test selection

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

N/A

4.4.2 Supplementary / Preparation of smears

N/A

4.5 Culture and investigation

N/A

4.6 Identification

N/A

4.7 Antimicrobial susceptibility testing

N/A

4.8 Referral for outbreak investigations

N/A

4.9 Referral to reference laboratories

The ectoparasites covered by this UK SMI are rare laboratory specimens in the UK and where appropriate should be forwarded to a reference laboratory for confirmation.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

London

The Diagnostic Parasitology Laboratory
London School of Hygiene and Tropical Medicine
Keppel Street
London, WC1E 7HT

OR

If in the Hays DX scheme:
PHE Malaria Reference Laboratory
DX 6641200
Tottenham Court Rd 92WC

Liverpool

The Clinical Diagnostic Parasitology Laboratory
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

OR

If in the Hays DX scheme:

Liverpool School of Tropical Medicine
Diagnostic Laboratory
DX 6966301
Liverpool 92L

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Glasgow

Scottish Microbiology Reference Laboratories
Glasgow Scottish Parasite Diagnostic and Reference Section
Level 5
New Lister Building
Glasgow Royal Infirmary
Alexandra Parade
Glasgow
G31 2ER

Northern Ireland

http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

N/A

5.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[44,45], or equivalent in the devolved administrations[46-49]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[46,47], Wales[48] and Northern Ireland[49].

References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	**Quality of evidence**
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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UK Standards for Microbiology Investigations

Abdominal organ transport fluid testing

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, National Infection Service, PHE

Technical | B 62 | Issue no: 1 | Issue date: 29.01.20 | Page: 1 of 12

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

Contents

Acknowledgments ....Error! Bookmark not defined.

Amendment table 4

1. General information 5

2. Scientific information 5

3. Scope of document 5

4. Background 5

5. Safety considerations 6

6. Investigation 6

7. Antimicrobial susceptibly testing 9

8. Referral for incident investigations 9

9. Referral to reference laboratories 9

Appendix: ODT rapid alert form 11

References 12

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	-/29.01.20
Issue number discarded	-
Insert issue number	1
Anticipated next review date*	29.01.23
Section(s) involved	**Amendment**

*Reviews can be extended up to five years subject to resources available.

1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

This UK SMI describes the examination of abdominal organ transport fluid (OTF) relating to abdominal organs destined for transplantation. It details the methodology for isolation and characterisation of potentially significant bacterial and fungal pathogens.

Isolation of microorganisms should not automatically trigger treatment. Treatment and management of positive results is a matter of clinical decision process and is outside the scope of this document.

This UK SMI should be used in conjunction with other relevant UK SMIs.

4. Background

Infectious complications are amongst the major causes of morbidity and mortality in patients undergoing solid organ transplantation, with proven donor-derived transmission of infection being an infrequent event. Contamination of the graft with subsequent recovery of micro-organisms from the OTF may represent a source of early post-surgical infection in transplant recipients. The OTF used to preserve the graft offers favourable conditions in which micro-organisms can survive; contamination may occur at different stages between retrieval and transplantation, including during multi-organ retrieval, back table manipulation of the organ in theatre, packaging and removal of the organ from the transport container.

The true clinical impact of microbiological contamination in abdominal organ transplantation remains unresolved, with isolation rates varying widely, from very low to up to 40%[1,2]. Whilst positive culture results seem to be infrequently linked to infectious complications with the same organism in recipients, well documented cases have been reported where significant recipient morbidity has ensued, particularly in the case of *Candida* spp[2-6].

The Organ Donation and Transplantation (ODT) directorate of NHS Blood and Transplant (NHSBT) is responsible for the procurement, characterisation and offering of organs from deceased donors in the UK. In 2017 it conducted a survey of microbiological practices in relation to microbiological culture of OTF amongst UK transplant centres, with a 52% response rate. The majority of responders (78.6%) indicated that microbiological culture of OTF has the potential to provide relevant information for patient management and that this information should be shared amongst centres receiving an organ from the same donor.

The audit also revealed that in the absence of guidance, there is wide variation in the methodology used and participants were keen to learn from the experiences of other

centres. Responders agreed that harmonisation of microbiological practices, including appropriate reporting of results, was highly desirable.

This UK Standard for Microbiology Investigations aims to address those gaps by developing an agreed methodology for culture of OTF, including the reporting of results, so as to contribute to improved and safer practices in abdominal organ transplantation. It does not prescribe treatment of isolates as a result of using this methodology.

It is hoped that with the harmonization of methodologies and practices, data can be collected to understand better and evaluate the role of microbiological culture of OTF.

5. Safety considerations

Containment Level 2.

6. Investigation

6.1 Culture of abdominal organ transport fluid

6.1.1 Specimen type

Abdominal organ transport fluid also known as perfusion fluid or transport perfusion fluid, is used to submerge abdominal organs during transportation between donor hospital and recipient transplant centres.

Abdominal organs (liver, pancreas and kidney) are packaged and cold stored by submersion in transport fluid. In the UK, two OTF types are used: University of Wisconsin (UW) solution and Soltran solution.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage[7]:

Type of specimen and sample registration

Samples should be clearly labelled as ‘Organ Transport Fluid’ and must be registered on the laboratory information management system under this specific specimen type.

Samples need to be logged and processed following local procedures in a manner that will allow the ODT Hub coordinator to link the results between donor(s) and recipient(s).

Optimal time and method of collection

For safety considerations refer to Section 5.

Collect specimen from fluid surrounding the organ immediately after the organ has been lifted from the transport bag for implantation.

Collect specimen into appropriate CE marked leak proof containers and place in sealed plastic bags.

Volume of material to submit for analysis[8]

The volume of transport fluid used differs depending on the size of organ being transplanted. A minimum of approximately 5% of the total volume in the organ transport bag should be used for analysis (ideally a minimum of 20mL).

Optimal transport and storage conditions

If not processed immediately following collection, specimens should be refrigerated and processed within 18 hours.

In accordance with ISO15189 standards, laboratories are responsible for monitoring the sample journey from collection through to commencement of processing to ensure specimen integrity and that samples are received within clinically-meaningful time-frames[8].

6.1.3 Laboratory processes (analytical stage)

Culture

Specimen processing

Centrifuge a minimum of 20mL of specimen (see 6.1) and use for inoculation of media plates:

- centrifuge in a sterile, capped, conical-bottomed container at 1200xg for 5mins
- remove all but the last 0.5mL of the supernatant using a sterile pipette
- re-suspend the deposit in the remaining fluid
- microscopy is not recommended
- inoculate each agar plate with the centrifuged deposit (see Q 5 – Inoculation of culture media for bacteriology) using a sterile pipette
- for the isolation of individual colonies, spread inoculum with a sterile loop

Table 1: Culture media, conditions and organisms

Standard media	Incubation			Cultures read	Target organism(s)
	Temp °C	Atmosphere	Time		
Blood agar	35-37	5-10% CO_2	40-48 hrs	16-24 hours and 40-48 hours	Any organism
Sabouraud agar	28-30	Air	5 days	Day 2 and Day 5	Moulds and Yeasts
CLED	35-37	Air	16-24 hours	≥16hr	Enterobacterales

Identification:

Refer to individual UK SMIs for organism identification.

Minimum level of identification in the laboratory

All organisms should be identified to species level.

Mixed growth is not common.

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to be a rapid and powerful identification tool for cultured isolates because of its reproducibility, speed and sensitivity of analysis and is available in most laboratories. Refer to UK SMI TP 40 - MALDI TOF MS test procedure.

6.1.4 Post-laboratory processes (reporting procedures)

Routine local laboratory reporting

Report:

- Clinically significant organisms with antimicrobial susceptibility results
- No growth of clinically significant organisms*
- No growth

** Identification should not be reported for organisms of no clinical significance.*

Communicating results to ODT

The communication process below is in addition to the routine reporting and communication of results between the laboratory and clinical teams, according to local protocols.

Local protocols should ensure that results are reported to and received from the ODT Hub with the timely involvement of clinical transplant and microbiology specialists.

It is acknowledged that local algorithms will determine whether communication of results to ODT Hub is done by the transplant team or the microbiology team.

Table 2: Organisms to be communicated to ODT Hub

Organism	When to send the Rapid Alert
Candida spp. Filamentous fungi *Staphylococcus aureus* Group A Streptococci	Communicate these organisms as soon as they are identified; antibiotic sensitivities to follow, when available
Enterobacterales *Enterococcus* spp. *Pseudomonas aeruginosa* Pyogenic streptococci (other than Group A)	Communicate organism ID and sensitivities together when both are finalised
Other organisms considered locally to be of potential clinical significance	At local laboratory discretion
No growth of clinically significant organisms' or 'No Growth'	Not to be communicated to ODT Hub

Mechanism of communication with ODT Hub

Refer to 'Appendix - ODT reporting form' for communication of results to ODT Hub, as detailed in Table 2 above.

All reports received by the ODT hub are forwarded promptly to all other transplant centres where organs from the common donors have been transplanted. These do not undergo clinical vetting and are cascaded immediately, for consideration by the recipient transplant centres.

7. Antimicrobial susceptibly testing

Refer to EUCAST guidelines.

Choice of agents to be tested for susceptibility should follow laboratory practice for isolates obtained from sterile sites.

Isolates requiring antimicrobial susceptibility testing

- *Candida* spp.
- Enterobacterales
- *Enterococcus* spp.
- Filamentous fungi
- *Pseudomonas aeruginosa*
- Group A streptococci
- Pyogenic streptococci (other than Group A)
- *Staphylococcus aureus*
- Other organisms considered locally to be potentially clinically significant

This UK SMI does not recommend susceptibility testing of isolates not deemed to be clinically significant.

8. Referral for incident investigations

As part of investigations, including possible transmission events and clinical incidents, laboratories are requested to assist the organ procurement organisation (ODT) by referring samples or isolates on request.

9. Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, or associated with a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Notes: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix: ODT rapid alert form

RAPID ALERT – ID & Sensitivities
Positive Organ Transport Fluid Result

Donor Information				
	ODT Number		Case number	
	Forename		Date of Birth	
	Surname			

Organ Transport Fluid Result		
	Which organ was transport fluid from: (for example left kidney, liver, pancreas)	
	Screenshot or copy of report attached? (please delete as appropriate): **Y / N** (If '**N**', please fill in details of isolate below)	
	Name of Organism(s)	
	Antibiotic sensitivities (Organism with asterisk on list below – sensitivities to follow)	*No need to fill in if attaching report*

Contact Details for Further Information			
	Microbiology	**Laboratory**	
		Contact name/role	
		Phone number	
	Transplant team	**RCPOC name/role**	
		Phone number	
		Name and role of person completing form	
		Date of completion	

Criteria for Communication of Results to ODT Hub according to organisms isolated

Organism	When to send the Rapid Alert
Candida spp. Filamentous fungi *Staphylococcus aureus* *Streptococcus pyogenes*	Communicate these organisms as soon as they are identified; antibiotic sensitivities to follow, when available
Enterobacterales Enterococcus spp. *Pseudomonas aeruginosa* Pyogenic streptococci (other than Group A)	Communicate organism ID and sensitivities together when both are finalised
Other organisms considered locally to be of potential clinical significance	At local laboratory discretion
No growth of clinically significant organisms' or 'No Growth'	Not to be communicated to ODT Hub

Email a completed copy of this form **immediately** to: odthub.operations@nhsbt.nhs.uk and they will disseminate to all relevant centres.

References

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UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

B 62 Abdominal organ transport fluid testing

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This publication was created by Public Health England (PHE) in partnership with the NHS.
Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

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Consultation: 16/07/2019 – 02/08/2019

Version of document consulted on: B 62dz+

Proposal for changes

Comment number	1		
Date received	16/07/2019	**Lab name/Professional body**	Sheffield Teaching Hospitals Microbiology department
Comment			
I have no problems with the methodology: I think mandating this culturing of fluids will result in unnecessary antibiotic treatment courses and logistical issues with transferring results. I don't disagree with candida/fungal culture - I don't think there is enough evidence to enable appropriate clinical management of any positive bacterial cultures.			
Evidence			
There is no good evidence that general bacterial culture of perfusion fluid gives better outcomes (excluding fungal culture).			
Financial barriers			
No.			
Health benefits			
More resistant organisms; more widespread use of broad spectrum antibiotics.			
Recommended action	**PARTIAL ACCEPT** The following sentence has been added to clarify: “Isolation of microorganisms should not automatically trigger treatment. Management of positive results is a matter of clinical decision process and is outside the scope of this document.”		

Comment number	2		
Date received	18/07/2019	**Lab name/Professional body**	Admin - Truro Microbiology
Comment			
This SMI is not relevant to our laboratory as we do not test for this or deal with transplant patients.			
Evidence			
Not completed.			

Financial barriers	
Not completed.	
Health benefits	
Not completed.	
Recommended action	**NONE**

Comment number	3		
Date received	19/07/2019	**Lab name/Professional body**	Freeman Hospital/NUTH
Comment			
RE: sections 7.4 and 8.2 and Appendix Why are mycobacteria mentioned as a potential pathogen yet there is no mention of culture on appropriate media? None of the media mentioned will grow mycobacteria. Either a note should be added to reflect this or (preferably), we should not list/mention this organism as a likely/potential target organism.			
Evidence			
Not completed.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** Mycobacteria has been removed from the document.		

Comment number	4		
Date received	26/07/2019	**Lab name/Professional body**	PHW Microbiology Cardiff
Comment			
Number 3.1 Specimen type and 4 background and 6 pre-laboratory processes a. What is the source of the OTF. Is it made by the centre? Is it sterile? Is there a process to use it? What is the transport bag for fluid collection? Is it and its outside sterile? Would a diagram help? 7.2 Culture and investigation			

<table>
<tr><td colspan="2">b. Differs from recent SMI for sterile fluid for fungi which is incubated for 21days. Previously where saboraurd was kept up for only 5days it was at a temperature of 26-30, not 35-37.</td></tr>
<tr><td colspan="2">Evidence</td></tr>
<tr><td colspan="2">Not completed.</td></tr>
<tr><td colspan="2">Financial barriers</td></tr>
<tr><td colspan="2">No.</td></tr>
<tr><td colspan="2">Health benefits</td></tr>
<tr><td colspan="2">No.</td></tr>
<tr><td>Recommended action</td><td>a. PARTIAL ACCEPT
A clarification to the specimen type has been added to reflect other names it is known by and what are the types of fluid used in the UK.
b. ACCEPT
Temperature changed to 28-30.</td></tr>
</table>

<table>
<tr><td>Comment number</td><td>5</td><td colspan="2"></td></tr>
<tr><td>Date received</td><td>28/07/2019</td><td>Lab name/Professional body</td><td>Salisbury District Hospital</td></tr>
<tr><td colspan="4">Comment</td></tr>
<tr><td colspan="4">Routine Laboratory Reporting, Section 8.1
a. I assume you mean to service users. It states not to report isolates which are not clinically significant. I would hope that this detail was still recorded somewhere. Also, given the organ is going into the patient and we can't see the future, surely at this point all isolates have the potential to be significant? The patient will be immunosuppressed so may have a higher risk of sepsis by an opportunistic pathogen, or even CNS.</td></tr>
<tr><td colspan="4">Evidence</td></tr>
<tr><td colspan="4">I just want it made clear that if the isolate is not reported to the service user, that it is still recorded on the microbiology LIMS, in case this information is needed later.</td></tr>
<tr><td colspan="4">Financial barriers</td></tr>
<tr><td colspan="4">No.</td></tr>
<tr><td colspan="4">Health benefits</td></tr>
<tr><td colspan="4">No.</td></tr>
<tr><td>Recommended action</td><td colspan="3">NONE</td></tr>
</table>

	Treatment and management of positive results is outside the scope of this document.

Comment number	6		
Date received	02/08/2019	**Lab name/Professional body**	UK Clinical Mycology Network
Comment			
Section 7.2 SAB slopes set up for 3 weeks and reads twice weekly.			
Evidence			
Experience from one of the UKCMN member laboratories: Over the past 3 years have had several cases of invasive Aspergillosis post solid organ transplantation (Renal and Liver) within 2 weeks of Transplantation, one of which was directly related to the graft destruction and haematogenous dissemination.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**NONE** As there is absence of evidence that this is a common situation.		

Comment number	7		
Date received	28/07/2019	**Lab name/Professional body**	Institute of Biomedical Science
Comment			
Section 6.1 Specimen collection, transport and storage a. Pg.6 “Sample needs to be logged and processed following local procedures in a manner that will allow linkage between donor and recipient” The appendix at the back suggests the laboratories will have a lot of information from the donor e.g. case number, full name – in practice will the sample be labelled as the donor or the recipient(?). If the concern is the management of the patient with the potentially infected organ then the OTF should be submitted for testing under the PID of the recipient – this will allow any microbiological advice to be issued based on the results for this patient. We are not aware of any LIMS that enable the donor name to be added – and this information could be sensitive – especially if from a cadaveric transplant – patient results and records can be subjected to FOI requests. It is suggested that the safest way to process these is to have a case number that is added to the recipient’s samples and logged in the			

LIMS like a reference number. The ODT Hub coordinator should be able to work back from the case number to find the donor information. It is suggested that statement should state: "Sample needs to be logged and processed following local procedures in a manner that will allow the ODT Hub coordinator to link the results between donor and recipient(s)" b. Section 7.1 Specimen processing/procedure Pg. 6 "Pellet a minimum of 20ml of specimen (see 6.3) and use for inoculation of media plates" This sentence lacks clarity, is pellet another term for centrifuge? There is no section 6.3. 7.2 Culture Moulds / Yeasts c. If the intention is to recover filamentous fungi then this should be stated in the target organism box – if the intention is to recover moulds the temp needs to be lower 28-30 and the incubation time extended. d. We are aware that "Enterobacterales" has replaced Enterobacteriaceae in other international guidance, would UK SMI consider this change? e. CLED agar is stated as one of the three agars to use with a suggested incubation time of up to five days. Manufacturers only validate CLED agar media for 18-24 hours incubation. We would suggest MacConkey agar as a suitable alternative that is validated for extended incubation. Five day incubation aerobically at 36°C can be problematic, especially in fan assisted incubators. User will need to ensure the plates remain viable and do not dehydrate. 7.3 Identifications f. Pg.7 "All organisms should be identified to species level, mixed growths are uncommon." There is no need to add "Organisms should be isolated and identified individually". 7.4 Antimicrobial susceptibly testing g. Group A Streps – not all are S.pyogenes – For consistency with other SMIs and PHE alerting Group A Strep is a better catch all. h. Whilst mycobacteria is stated as a desired target organism. Neither of the defined media are likely to grow mycobacteria as these typically require >72hrs incubation on blood agar or other specialised agar media to grow. The suggested length of incubation in the SMI is up to 48hrs which would not provide an adequate incubation period.
Evidence
Not completed.
Financial barriers
Not completed.
Health benefits
Not completed.

Recommended action	a. **ACCEPT** Sentence changed to: "Sample needs to be logged and processed following local procedures in a manner that will allow the ODT Hub coordinator to link the results between donor and recipient(s)". b. **ACCEPT** Word pellet changed to centrifuge and 6.3 changed to 6.1. c. **ACCEPT** Temperature changed to 28-30. d. **ACCEPT** Changed to Enterobacterales. e. **ACCEPT** Time of incubation for CLED was changed from 5 days to 16-24 hours. f. **ACCEPT** "organisms should be isolated and identified individually" has been removed. g. **ACCEPT** Changed to Group A streptococci and Pyogenic streptococci (other than Group A). h. **ACCEPT** Mycobacteria has been removed from document.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 3			
Date received	16/07/2019	**Lab name/Professional body**	Freeman Hospital
Health benefits			
No.			
Date received	24/07/2019	**Lab name/Professional body**	Healthcare Infection Society
Health benefits			
Not completed.			
Date received	26/07/2019	**Lab name/Professional body**	Microbiology Laboratory, Western Health & Social Care Trust

Health benefits
No.

<table>
<tr><td rowspan="4"> </td><td>NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>1 of 8</td></tr>
<tr><td rowspan="3">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Effective from</td><td>October 2019</td></tr>
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This is a local SOP and only applies to the Paediatric Haematology/Oncology Patient Cohort for the Duration of their stay in ward 6a

SOP Objective

To monitor the risk of environmental organisms in the paediatric haematology-oncology patient cohort.

KEY CHANGES FROM THE PREVIOUS VERSION OF THIS SOP

- None - New Document

Document Control Summary

Approved by and date	Women's and Children's Clinical Governance Group
Date of Publication	--
Developed by	Gram negative bacteraemia Incident Management Group Health Protection Scotland
Related Documents	NHSGGC Hand Hygiene SOP NHSGGC SICPS SOP Microbiology SOP Air Sampling Microbiology SOP Water Testing DME SOP obtaining water samples.
Distribution/ Availability	NHSGGC Infection Prevention and Control Policy Manual and the Internet www.nhsggc.org.uk/your-health/public-health/infection-prevention-and-contol
Lead Manager	Board Infection Control Manager
Responsible Director	Director Women & Children's Services

<table>
<tr><td rowspan="4"> </td><td>NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>2 of 8</td></tr>
<tr><td rowspan="3">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Effective from</td><td>October 2019</td></tr>
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</table>

Contents

1. Responsibilities 3

2. Air Sampling

3. Environmental Sampling

4. Water Sampling

<table>
<tr><td rowspan="4"> </td><td rowspan="2">NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>3 of 8</td></tr>
<tr><td>Effective from</td><td>October 2019</td></tr>
<tr><td rowspan="2">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Review date</td><td>April 2020</td></tr>
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1. **Responsibilities**

Healthcare Workers (HCWs) must:

- Inform a member of the Infection Prevention and Control Team (IPCT) if this SOP cannot be followed.

Clinicians and Microbiologists must:

- Review reports and provide clinical interpretation (micro)
- Act on advice of microbiology (clinicians)

Microbiology Laboratory

- Escalate exceptions with water samples to IPCT
- Respond to ICD request for environmental samples (water, air, environment*)
- Escalate exceptions with air/environment to IPCT

Estates and Facilities Management

- Escalate exceptions with water samples to IPCT
- Respond where appropriate to request for samples
- Inform a member of the Infection Prevention and Control Team (IPCT) if this SOP cannot be followed

Managers must:

- Ensure that staff are aware of the contents of this SOP.
- Support IPCT/EFM s in following this SOP.

*Infection Control Nurses may carry out environmental samples at the request of IMT or ICD.

2. Air Sampling

Background	Ward 6a is categorised as a general ward area. In terms of air flow pressures this type of ward has been designed to meet the specification required for a general ward area and therefore no pressure cascade is required as per appendix 1 table A1 SHTM 0301 part A, however this ward was modified prior to occupation to make the ward nominally positive from the room to the corridor and therefore from clean to dirty in October 2018, however this is reliant on staff ensuring that doors are closed as much as possible. This is not a specially ventilated ward therefor there is an expectation that sampling will be positive. In addition to this modification mobile HEPA filters are located in rooms, corridor and ancillary areas. These are designed to reduce the numbers of microorganism in the air. Air sampling has been performed on a regular basis (twice weekly) to ensure that the HEPA filters are working in the rooms. Prior to sampling the room should be empty and have not been reciently cleaned. Air sampling in 5 rooms should be undertaken each time if possible. There is no requirement to sample corridors or communal areas. There is no requirement as such to air sample and no agreed standards or guidance for interpretation of air sampling for general ward areas or indeed UK BMT units. Many units, including BMTU do not conduct air sampling. It is therefore unclear what interpretative criteria to apply and what actions to take when results are elevated. Air sampling results and fungal culture take 7 days to initial identification and a further 7 days for species identification. Clinical assessment of high risk patients (neutropenic patients) and their subsequent placement in specially ventilated areas, e.g 4B should be completed by clinical staff within the ward.

<table>
<tr><td rowspan="4"> </td><td rowspan="2">NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>5 of 8</td></tr>
<tr><td>Effective from</td><td>October 2019</td></tr>
<tr><td rowspan="2">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Review date</td><td>April 2020</td></tr>
<tr><td>Version</td><td>1</td></tr>
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</table>

Recommendation

The Centre for Disease Control in the USA published Guidelines for Environmental Infection Control in Health-Care Facilities in 2003.

This SOP proposed to use recommendation for environmental standards in ITU areas as a proposed measurement for interpreting sampling results in ward 6a.

Only rooms and shower areas should be sampled.

10 rooms should be randomly sampled every 4 weeks. Microbiology have protocols in place with regards to methodology.

Table 1

Hazard / Indicator	**Result**	**Interpretation**	**Action**
Total bacterial	counts < 50 cfu/m ^{3}	Satisfactory	None
	counts > 50 cfu/m ^{3}	unsatisfactory	Take room out of use, clean thoroughly and re-test
Aspergillus	Counts < 5 cfu/m ^{3}	satisfactory	None
	counts > 5 cfu/m ^{3}	unsatisfactory	Take room out of use, clean thoroughly and re-test

Any obvious ingress of water should be reported to the IPCT and the SCN within the unit. Only cfu and aspergillus should be sampled for.

3. Environmental Sampling

Background	Increase in the incidence of gram negative and environmental microorganisms.
Recommendation	**Environmental sampling should be instructed by the ICD and these factors should be considered before starting the environmental surface sampling:** • Background information (sampling objective (a) outbreak (b) renovation). • Determine the target microorganism (s) in case of outbreaks. • Location of surfaces to be sampled. • Method of sample collection and the appropriate equipment or supplies for each task. **Method** • Arrange all the supplies and equipment needed. - Sterile non powdered vinyl gloves (should be replaced after each sample). - A sterile non cotton or cotton swabs with transport media or dry swabs for moistened areas. • Wear the non powdered sterile gloves. • Remove a sterile, cotton swab from the package.

<table>
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Moisten the swab with (sterile water, peptone water, or transport media).
Wipe the surface to be sampled by touching the cotton tip to the surface and rotating and rubbing back and forth for the deep irregular areas.
For the flat surfaces, the suggested sampling technique is to make a vertical S – strokes to cover an area of 10 x 10 cm².
Place the swab in its tube taking care not to touch the tip or the shaft of the swab.
Label tube containing the swab with date, specific location, and facility).
Transport the samples to laboratory for analysis as soon as possible.
Return laboratory results to appropriate ICD who will advise on further actions.

</td></tr>
</table>

4. Water Sampling

<table>
<tr><td>Background</td><td>Dosing with chlorine dioxide is in place in QEUH/RHC. In addition PAL filters are present on all outlets in areas where high risk haematology/oncology patients are located.</td></tr>
<tr><td>Recommendation</td><td>This will be carried out by an external contractor as advised by the:

NHSGGC water technical group as advised by external experts.
Board Water Safety Group
Authorising Engineer
In response to an outbreak or incident

Results will be reviewed at water technical group. Exceptions should be sent to Consultant Public Health Medicine and Infection Control Doctor for the site.</td></tr>
</table>

<table>
<tr><td rowspan="2"> </td><td>NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>8 of 8</td></tr>
<tr><td rowspan="3">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Effective from</td><td>October 2019</td></tr>
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Evidence Base

Guidelines for Environmental Infection Control in Health-Care Facilities Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC) Prepared by: Lynne Sehulster, Ph.D.1, Raymond Y.W. Chinn, M.D.21Division of Healthcare Quality Promotion, National Center for Infectious Diseases HICPAC member, Sharp Memorial Hospital, San Diego, California 30333, 2003.

Guidelines for the Collection and Interpretation of Results from Microbiological Examination of Food, Water and Environmental Samples from the Hospital Environment Caroline Willis, David Lamph, Kathy Nye, Elizabeth Youngs, Heather Aird, Andrew Fox and Susanne Surman-Lee *Health Protection Agency Food Water and Environmental Microbiology Network* .June 2010.

<table>
<tr><td rowspan="4">NHS Greater Glasgow and Clyde</td><td>NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>1 of 7</td></tr>
<tr><td rowspan="3">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Effective from</td><td>.. 2019</td></tr>
<tr><td>Review date</td><td>...</td></tr>
<tr><td>Version</td><td>...</td></tr>
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This is a local SOP and only applies to the Paediatric Haematology/Oncology Patient Cohort

SOP Objective

To minimise the risk of environmental organisms in the paediatric haematology-oncology patient cohort.

KEY CHANGES FROM THE PREVIOUS VERSION OF THIS SOP

- None - New Document

Document Control Summary

Approved by and date	Women's and Children's Clinical Governance Group
Date of Publication	--
Developed by	Gram negative bacteraemia Incident Management Group
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Contents

	NHS GREATER GLASGOW & CLYDE CONTROL OF INFECTION COMMITTEE POLICY	Page	2 of 7
		Effective from	.. 2019
	STANDARD OPERATING PROCEDURE (SOP) **Environmental Monitoring in the Haematology Oncology Unit Ward 6a**	Review date	...
		Version	...
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1. Responsibilities 3
2. Air Sampling
3. Environmental Sampling
4. Water Sampling

<table>
<tr><td rowspan="2"> </td><td>NHS GREATER GLASGOW & CLYDE
CONTROL OF INFECTION COMMITTEE POLICY</td><td>Page</td><td>3 of 7</td></tr>
<tr><td rowspan="3">STANDARD OPERATING PROCEDURE (SOP)
Environmental Monitoring in the Haematology Oncology Unit Ward 6a</td><td>Effective from</td><td>.. 2019</td></tr>
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Recommendation	The Centre for Disease Control in the USA published Guidelines for Environmental Infection Control in Health-Care Facilities in 2003. This SOP proposed to use recommendation for environmental standards in ITU areas as a proposed measurement for interpreting sampling results in ward 6a. Only rooms and shower areas should be sampled. 10 rooms should be randomly sampled every 2 weeks.

Table 1

Hazard / Indicator	Result	Interpretation	Action
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Wear the non powdered sterile gloves.
Remove a sterile, cotton swab from the package.
Moisten the swab with (sterile water, peptone water, or transport media).
Wipe the surface to be sampled by touching the cotton tip to the surface and rotating and rubbing back and forth for the deep irregular areas.
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Place the swab in its tube taking care not to touch the tip or the shaft of the swab.
Label tube containing the swab with date, specific location, and facility).
Transport the samples to laboratory for analysis as soon as possible.
Return results to appropriate ICD.
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4. Water Sampling

Background	Dosing with chlorine dioxide is in place in QEUH/RHC. In addition PAL filters are present on all outlets in areas where high risk haematology/oncology patients are located.
Recommendation	This will be carried out by an external contractor as advised by the: NHSGGC water technical group Board Water Safety Group Authorising Engineer In response to an outbreak or incident Results will be reviewed at water technical group or by ICD during incidents or outbreaks.

Evidence Base

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Environmental Monitoring	
LABORATORY PROCEDURE	
NUMBER / VERSION	LP089
DATE OF ISSUE	17/03/2015
REVIEW INTERVAL	2 Years
AUTHORISED BY	Karen Cullen
AUTHOR	K Cullen/B Lavery
COPY 1 of 1	Master file in Q-Pulse
LOCATION OF COPIES	1. Environmental Laboratory 2. Environmental Box for on site sampling

DOCUMENT REVIEW HISTORY
All review / revision details are available in Q-Pulse

Date	Amendment	Initials

Scope

This Standard Operating Procedure (S.O.P) describes the process for air sampling using the Aquaria micro flow, Contact plates and the Hach Met One 3400 particle counter. All state registered BMS staff should be able to perform the tasks within this S.O.P. Trainees should always work under supervision when carrying out tasks within the S.O.P.

Two members of staff MUST at all times be in attendance when monitoring sites i.e. 1 BMS and 1 CSW or 2 BMS staff. BMS staff must always be present when environmental monitoring is being done.

Introduction

Environmental monitoring involves Air Sampling, Particle Counting and the use of Contact Plates.
The analysis of Air and Particle Counting involves the use of specialist equipment the HACH MET ONE 3400 and the AQUARIA MICRO-FLOW.
Contact plates are also used.
It is necessary to sample air to ensure air supply has satisfactory low concentration of airborne bacteria.

CONTACT PLATES

AQUARIA MICROFLOW

HACH MET ONE 3400 PARTICLE COUNTER

Safety Considerations

RISK ASSESSMENT	REFERENCE	HAZARD	PRECAUTIONS
Safety Manual	SM001	General	GLP
Working in the water lab	R107	Physical	Heat and electrical safety awareness
General Culture	R011	Biological	Hand washing

Use of Equipment for Air Sampling

<u>Aquaria Micro flow</u>

The Aquaria Micro flow has been developed for air bio contaminants sampling in critical places (e.g. clean rooms, theatres and H.E.P.A areas e.g. immuno-compromised patients in single ward). Micro flow allows verifying and quantifying microorganism presence. In this way you can value the exposure or you can identify the source in order to put in place corrective actions.

The Aquaria Micro flow battery should be charged prior to use by using the charger and plugging into the electrical socket.

Stand by: BATT CHARGING is displayed.
Display will read as Battery 100% when fully charged

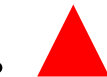
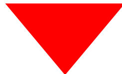
To Use:

- Remove lid from Aquaria Micro flow
- Wipe the perforated plate and metal tabs that are exposed with an alcohol wipe.
- Select a TSA or a Saboraud media plate depending on whether fungal or bacterial monitoring is required
- Place the media plate between metal tabs which will secure the plate into position and tighten the lid of the Micro flow.
- Place the air sampler at designated position within the area to be monitored, these will be normally be inside cabinets in use and areas within site that have been agreed
- Start the Aquaria Micro flow by pressing ON/OFF

The Aquaria Micro flow is pre- set programmed to monitor 500 ml of air for 4 minutes at a flow rate of 120 l/min

- To start the Aquaria Micro flow Press

- The screen will display Program sampling and a Time Countdown begins decreasing from 4 minutes to Time 0 (finish)
- Alarm bleeps on the Aquaria Micro flow when the time is complete by a flashing RED light
- Press ENTER to escape
- Remove culture plate from Aquaria Micro flow- the agar should be depressed with 'tram lines'
- Start process again for additional culture plates, wiping the perforated lid and metal tabs in between each culture plate placed within tabs.

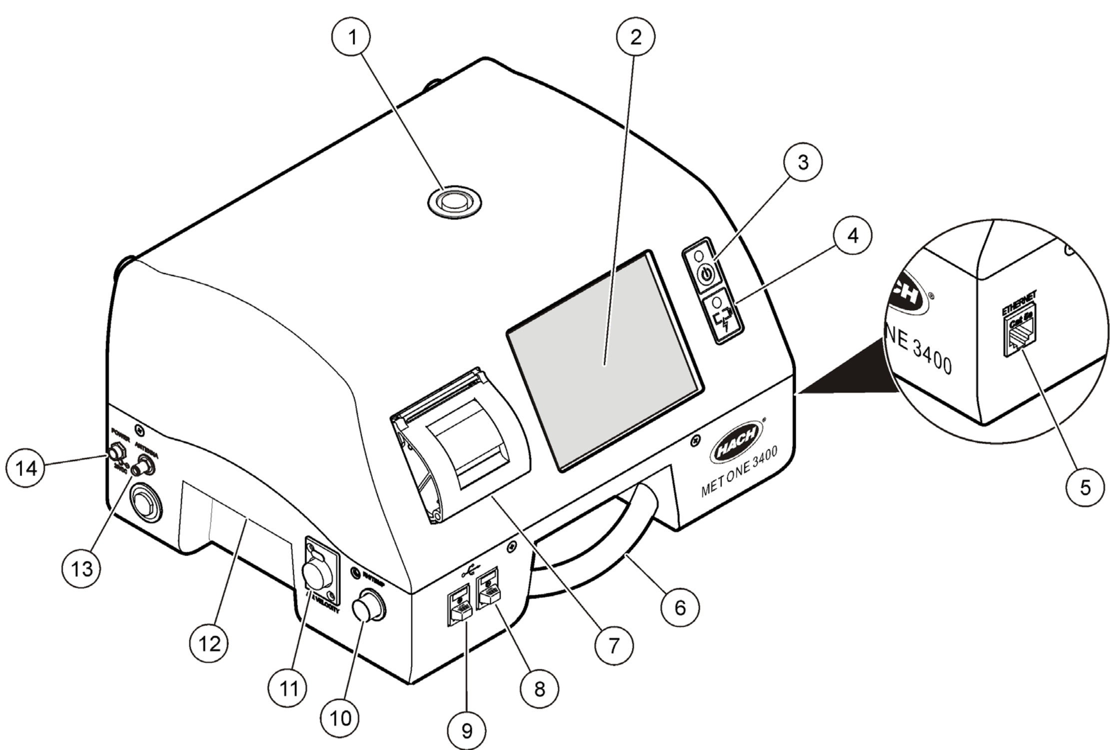
- If Programme requires to be altered - Press Prog.
- Can select Programmed change, manual change or Sequential change by pressing
-   for Up / Down

- Press Enter to select programme if required to change Volume of Air required to monitor, Flow Rate and Delay start if required

HACH MET ONE 3400 PARTICLE COUNTER

Hach Ultra Analytics particle counters use a laser-diode light source and collection optics for particle detection. Particles scatter light from the laser diode. The collection optics focuses the light onto a photo diode that converts the bursts of light into electrical pulses. The pulse height is proportional to the particle size. Pulses are counted and their amplitude is measured for particle sizing. Results are shown as particle counts in the specified size range (differential count mode) or as total particle count counts (Cumulative count mode).

The MET ONE 3400 is operated by use of a TFT colour touch screen located on the front panel of the unit. All commands are executed through the touch screen.



1 Sample intake nozzle
2 Touch screen
3 Power button
4 Battery status indicator
5 Ethernet connector
6 Handle
7 Printer
8 USB host connector
9 USB client connector
10 Relative humidity and temperature probe connector
11 Air velocity probe connector
12 Handle
13 Wireless antenna connector
14 Power connector

Particle counter navigation
The functions of the particle counter are accessed from the Counter Navigation screen

Icon	Function	Description
	Sample	Measures particle counts
	Historical	Review measurement results in the Buffer, print
	Export	Output file as comma separated value (CSV), Tab separated, or PortAll files. Refer to the CD for information
.	Printer	Print sample data as hard copy
	Locations	Add/Edit/remove areas, copy location
		Settings edit location settings, edit alarms for Specific locations.
	Group	Load/add/edit a group, delete a group.

	System	Time/Date. Sleep time/backlight timeout, set Logon requirements, set sounds for alarms, Manage users, set units for flow rates, and Manage the data buffer.
	Sizes	Add/edit/delete a size (optional).
	Test Wizard	Test and report wizard for ISO, EU-GMP, FS or BS classification compliance.
	Return	Return to the previous screen or menu.

Operation

Charge the batteries in the particle counter
Batteries in the 3400 will begin to charge when the AC power cord is connected. A complete charge in the instrument takes approximately 10 hours. The battery is considered to be fully charged when they display shows the charge between 95% and 100%.

Battery LED colour indications:

LED state LED colour Battery status Charge status

Flashing Orange Low power not charging
Flashing Green Low power charging
Solid Green Charged charging

Log on to the particle counter

Prerequisites

- Remove the RED protective cap from the inlet tube on the counter
- Start the system.
- Activate the backlight with a finger or stylus if needed.
- Counter Navigation Touch screen is displayed
  On the Sample screen, push the location name. Select the new Name and confirm.
  On the Sample screen, push the button to increment the location, or push the button to decrement the location.
- Example BMT Gartnavel , this then opens up into areas within BMT Gartnavel for selection
- To add or remove a location select appropriate tab displayed on Touch screen.
- Press to go back to Counter Navigation screen

To Run a Particle Count

- On the Counter Navigation screen, push **SAMPLE**.
- To start the particle count, push the **RUN** button. The **RUN** button will change to a **STOP** button while the count is measured.
- The Particle counter is set to perform 3 x 1 min sampling and a Cumulative average is calculated and printed.
- When the count measurement is complete, the test will stop automatically.
- The Cumulative average at 0.5µm is the figure is routinely used unless otherwise instructed.

After a complete particle count measurement, the number of particles measured will show on the screen and be stored as data. Other configured parameters, such as relative humidity, temperature and air velocity will be shown and stored in data.

How to see historical data during the particle count

Historical sample data can be seen at any time during the particle count cycle.
On the Sample screen, select the **ARROW** button.
Select the **HISTORICAL DATA** icon to see the data.

How to use the Print Centre

About the Print Centre

To avoid damage to the printer, do not operate the printer without paper. If the particle counter must be used
Without paper, be sure to set the print mode to "None".
The particle counter has a built-in printer. The Print Centre screen is accessible from the:

- Counter Navigation screen
- Historical screen
- System Diagnostics screen
- Test/Report Wizard screen
- Area/Location Setup screen

On the Print Centre screen the user can:
- Set automatic print functions
- Print buffer records or count averages

Print records manually

The buffer holds 5000 records maximum. The Print Centre can print the entire buffer or the average of count cycles.
To print records manually:

On the counter Navigation screen, push **PRINTER.**
On the Print Centre screen, select the print option for the data.
The data will begin to print.
To cancel the print job, push **CANCEL PRINT**.
To return to Counter Navigation, push **RETURN**.

Maintenance

W A R N I N G

Multiple hazards. Do not disassemble the instrument for maintenance or service. If the internal components must be cleaned or repaired, contact the manufacturer.

Set the count to zero

Do this procedure after unexpectedly high particle counts. This procedure will verify that the particle counter works correctly and will remove residual particles

.

Put the zero-count filter on the intake tube.
Turn on the unit and log in if needed.
Push **SAMPLE**.
Push **RUN**.
Repeat the process until the particle counts return to zero.

Air Sampling/ Particle Counts/ Contact plates

BMS and CSW may be asked to go off site to monitor areas within GGC. Taxi transfer should be used to go to another location within GGC. Authorisation information as regards ordering a taxi can be obtained from the Technical Manager or Site Manager.

The areas currently monitored monthly are:

- Gartnavel General Hospital Haematology (GGH Haematology Stem Cell Laboratory)
- Bone Marrow Transplant Unit B8, B9 at Beatson Gartnavel General
- Assisted Conception Service at GRI

Other areas which may be monitored on an ad hoc basis are

- Theatres at request of Infection control Consultant
- Any other sites under Greater Glasgow and Clyde requested by Infection Control Consultant

GGH General Hospital Haematology

Address: 21 Shelley Rd, Level 2, Stem Cell Lab, Haematology, Gartnavel Hospital

When arriving at GGH Haematology to do Air Sampling, access to Haematology dept is by Controlled door entry. Press buzzer for access. Staff at GGH should always know to expect you by prior arrangement.
The Stem Cell Processing lab is the only area monitored within Haematology.

Particle Counts:

- Test the cabinet nearest the door (MC5177)
- Furthest from the door (MC5176)
- Bench (air in room)
- Outside count

Air Samples: The sample volume is 500 litres

- Test the cabinet nearest the door (MC5177)
- Furthest from the door (MC5176)

For each sample site:

- Incubate 2 x Sabouraud at (22°C and 30°C for 7 days)
- Incubate 1 x TSA at 30 °C for 7 days

Contact plates:

- Test areas are work surfaces in cabinets nearest the door
- Work surfaces in cabinets furthest away from the door.

For each sample site:

- Incubate 2 x Sabouraud at (22°C and 30°C for 7 days)
- Incubate 1 x TSA at 30°C for 7 days

Baxter's Hematron Bag Sealer:

Bag sealers are located at the side of each cabinet named as Baxter's Hematron #1 and #2. They can be switched on at the back of Hematron.

Using a sterile Trans swab, swab top and bottom of the handle of each Hematron

- Before heating
- After heating - press blue button to heat

Inoculate

- CBA at 35°C for 48HR
- Sabouraud at 30°C for 7 days
- TSA at 30°C for 7 days

Bone Marrow Transplant Unit B8, B9 at Beatson Gartnavel General

Monitoring of the Beatson B8, B9 Unit requires prior arrangement before arriving at the Unit. Staff from Microbiology MUST presents themselves to Beatson staff at the Unit upon arrival.

All staff is to be aware that laboratory coats are not to be worn for work carried out in Bone Marrow Transplant Unit (BMTU).

- Plastic aprons are to be used on arrival at the ward.
- Alcohol gel to be used on hands on entering ward.

Particle Counting- Before leaving the department a particle count of the outside air is to be taken. This provides a comparison with the count from the room sampled.

- Eight rooms are tested at random in each unit of the 4th floor B8,B9
- Record the results in the daily worksheet
- Document on sheet if any patients or others in the room at the time or if cleaners are in the area.

Air Sampling - is carried out on a monthly basis

- Eight rooms are tested at random in each Unit of the 4th Floor B8,B9
- Incubate 2 x Sabouraud at (22°C and 30 °C) for 7 days

Assisted Conception Unit at GRI (ACU)

Monitoring of the Assisted Conception Unit requires prior arrangement before arriving at the unit. It is usually requested by the unit through the Infection Control Microbiologist.
Microbiology staff will be escorted to the staff changing lockers by ACU staff where they will be requested to change into scrubs.

All staff is to be aware that this is a highly sensitive area, staff for Microbiology should always check with ACU that it is appropriate at that particular time to enter certain areas, namely procedure rooms, as work with a patient may be ongoing.

ACU staff will direct Microbiology staff to the Main Lab for testing to begin.

Please refer to ACU Floor plan for highlighted areas requiring testing:

Main Lab Area:

1. Cabinet 1
2. Cabinet 2
3. Cabinet 3
4. Cabinet 4
5. Cabinet 5
6. Cabinet 6

10. Main lab area left
14. Main lab area right

Adjoining Andrology Lab (to Main Lab)

7. Cabinet 7
8. Cabinet 8

19. Area within Andrology lab

Sterile Procedure Room 1

25. Scrub Area
28. Bench area

Procedure Room 2

33. Corner of room

Lobby

23. Area opposing sink

Particle Counts- Before leaving the department particle counts of the outside air is to be taken.

- Each area mentioned above require particle counts

Air Sampling – Each area mentioned above requires

- Sabouraud 30°C for 7 days
- Sabouraud 22° C for 7 days
- TSA 30° C for 7 days.

Theatres

There is sufficient evidence to support the undertaking of microbiological air sampling:

1. As part of the commissioning of an operating theatre
2. After any major restructuring refurbishment including alterations to the fabric of the theatre or changes to the ductwork distribution that may affect airflow to or within the theatre suite. (Minor changes to ductwork may not necessitate sampling – discuss with Infection Control)
3. As deemed necessary by Infection Control e.g. as part of an outbreak investigation

Procedure for Sampling

- The theatre should have had an in-depth clean and be thoroughly clean and dust free at least one hour before sampling.

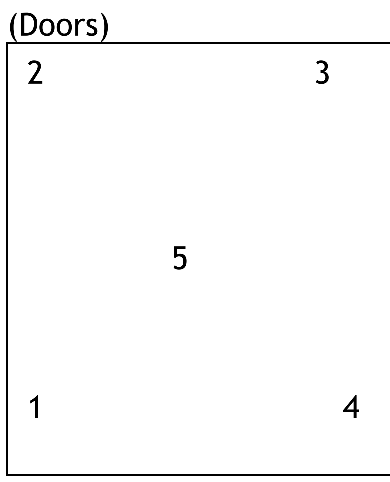
    - ❖ It is crucial that no- one should have entered the theatre in the one hour prior to air sampling

- The air handling unit should have been operating at normal flow rates continuously for at least 24hours before sampling in a new theatre and for one hour in an existing theatre
- The air sampler should be cleaned before use with 10% Distel on the outside and alcohol wipe on the inner plate and metal tabs
- It should run briefly before the agar plate is loaded to blow any contamination out of the sampler
- Air Sampling is carried out in 2 positions using TSA plates with a sample size of 500L. Set time delay at 15 minutes to allow enough time to exit room and air flow to settle
- Incubate TSA at 37° C for 2 days
- HTM 2025 Commissioning of Theatres gives a pass at less than 10 colony forming units in one cubic metre of air

    - ❖ PLEASE INFORM INFECTION CONTROL CONSULTANT IF RESULT IS > 10 cfu/m^3

Procedure for Particle Counts

- Prior arrangements are made with theatre sister to make sure air flow cabinet is left running. Alternatively switch on to operational speed
- Prior to entering theatre area, theatre greens, overshoes and head covering must be worn
- Each theatre is tested at 5 specified sites underneath micro flow cover
- Each test location should be numbered 1-5 , and recorded on the print out



- Printout is inspected and noted as a pass or fail

- Result interpretation- each theatres particle count should be either no recorded particles or < 100 particles at 0.5 µm

  - **PLEASE INFORM INFECTION CONTROL CONSULTANT OF ANY RESULT OUTWITH THIS RANGE**

Booking in procedure

Ensure the specimen details match the request form, and sufficient information is recorded on the form to issue a report.
Record date & time of receipt of the specimens using the Seiko unit.

- Book in samples using designated air sample laboratory numbers
- Note each sample / site is given an individual lab. Number
- Record lab. numbers in Air Sampling day sheet LF 218
- Incubate the culture plates accordingly
- Outstanding request forms are kept at the front of the folder
- Particle (Cumulative Average at 0.5 µm) and Culture results are recorded onto the Air Sampling day sheet LF218 and then transcribed onto the request form when ready to report result.

Interpretation of Results

Air Sampling:
For all areas sampled except Stem Cell lab- report any growth of fungus to Infection control consultant.
For stem cell lab - report any growth of fungus to stem cell lab Quality manager (QM) and technical manager (TM)
Operating theatres >10cfu/m³ - report to Infection control consultant.

Particle Counts:
Assisted Conception Suite and Stem Cell Processing Lab
Acceptable counts are listed below. Any counts out with these ranges report to Infection control consultant for ACS and QM/TM for stem cell lab.

Grade	At rest particle count		In operation particle count	
	≤0.5 µm	5 µm	≤0.5 µm	5 µm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Undefined	Undefined

Beatson B8/9
> 1000 particles at 0.5um in any room – report to Infection control consultant.

Operating theatres
> 100 particles at 0.5um – report to Infection control consultant

Contact Plates:

Assisted Conception Unit/Stem cell lab
Acceptable results below – out with these contact Infection control consultant for ACS and QM/TM for stem cell lab

Grade	Settle plates (90mm diameter) cfu/plate	Contact plates (55mm diameter) cfu/plate
A	< 1	< 1
B	5	5
C	50	25
D	100	50

North Glasgow Microbiology	LP089
	Authorised by Karen Cullen
Issued 17/03/2015	Author K Cullen/B Lavery

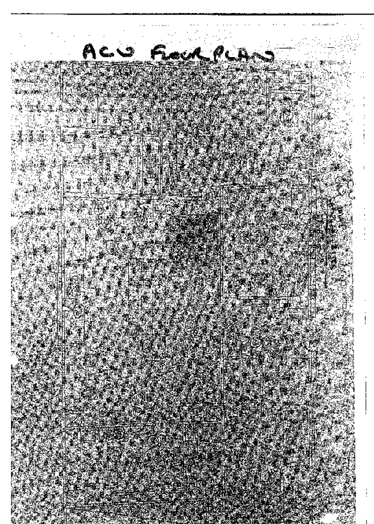
Environmental Monitoring Chart

Air Sampling						
Location	Sab 30°C 7 days	Sab 22° C 7 days	TSA 30 °C 7 days	CBA 35°C 48 Hr	Particle Counts	Contact Plates
GGH Haem Stem Cell Lab						
Cabinet 1					**** see below	*** see below
Cabinet 2					****	***
Bag Sealer before heating						
Bag Sealer after heating						
Beatson B8, B9 4th Floor						
(8 rooms tested at random)						
4 rooms B8					****	
4 rooms B9					****	
Assisted Conception Service						
see floor plan for site specific					****	Cabinets if required
Theatres 2 positions			2 days		****	

*** Contact plates Sab 22°C Sab 30°C TSA 30°C for 7 days

**** Outside particle count and Overall Room

N.B. CBA at 35°C only as requested by Infection Control Microbiologist



Double click Mouse to Open

FLOOR PLAN OF ASSISTED CONCEPTION SERVICE

Environmental Monitoring

LOCATION OF COPIES	1. Environmental Laboratory 2. Environmental Box for on-site sampling

DOCUMENT REVIEW HISTORY
All document details are available in Q-Pulse

Date	Amendment	Initials
Further Amendments will require the document to be updated to the next version, incorporating all previous listed amendments		

1. Purpose of examination

Environmental monitoring involves Settle Plate Analysis, Contact Plate Analysis, Air Sampling & Particle Counting.

Settle Plates
This involves the use of a selection of agar plates, details follow below. This ensures that the specific working environment is clear of potential contamination that could affect the processes that are being carried out in that area.

Contact Plates
Small agar plates placed in contact with specific surfaces and incubated according to their requirements. These are additional aids in environmental monitoring of these surface areas.

Air Sampling
This involves the use of specialist equipment the AQUARIA MICRO-FLOW. This is developed for air bio contaminants sampling in critical places (e.g. clean rooms, theatres and H.E.P.A areas such as immuno-compromised patients in single ward). Micro flow allows verifying and quantifying of microorganism presence. In this way you can value the exposure or you can identify the source in order to put in place corrective actions.

Particle Counting
This involves the use of specialist equipment the HACH MET ONE 3400. Hach Ultra Analytics particle counters use a laser-diode light source and collection optics for particle detection. Particles scatter light from the laser diode. The collection optics focuses the light onto a photo diode that converts the bursts of light into electrical pulses. The pulse height is proportional to the particle size. Pulses are counted and their amplitude is measured for particle sizing. Results are shown as particle counts in the specified size range (differential count mode) or as total particle count counts (Cumulative count mode).

2. Principle and method of the procedure

This SOP details the procedure to check the level of environmental condition in various locations using the above mentioned processes. Bacteria, Fungi & Particle Counts are recorded and reported to the Infection Control Doctors who monitor each area for acceptable levels. It is necessary to sample air to ensure air supply has a satisfactory low concentration of airborne bacteria.

State registered BMS & CSW perform the tasks within this SOP. Trainees should always work under the supervision of a qualified BMS when carrying out tasks within the SOP.

Two members of staff MUST at all times be in attendance when monitoring sites i.e. 1 BMS and 1 CSW or 2 BMS staff. BMS staff must always be present when environmental monitoring is being done.

3. Type of Sample

Air Sample
Particle Count
Settle Plate
Contact Plate

4. Required Equipment and Reagents

Particle Counter

Air Sampler
Agar Plates : TSA, Sabouraud, Blood Agar, GVPC

5. Environmental and Safety Controls

Risk assessment	Reference	Hazard	Precautions
Safety Manual	Refer to local safety manual	Biological	General lab practice
Working in the water/environmental lab	R537	Biological, Physical,	General lab practice, PPE
Working with ACDP hazard group 2 Organisms	R529	Biological	Wear PPE - Lab Coat
Off Site Environmental Testing	R538	Physical	Manual Handling

6. Calibration Procedures

- Particle counter calibrated yearly by Beckman Coulter
- Air Sampler calibrated yearly by Protrol

7. Procedural Steps

7.1. Sampling Procedure

Settle Plates

GRI Laboratory environmental settle plates -Refer to LP534 for procedural steps
External locations may submit settle plates for investigation. Incubate as specified under location sections below.

Contact Plates

- Sabouraud and/or TSA agar
- These plates are opened and the agar side is pressed gently onto the surface i.e. contact is made with the areas specified below for one or two seconds
- The plates are closed, brought back to the lab and incubated according to their requirements

External locations may submit contact plates for investigation. Incubate as specified under location section below

Air Sampling

The Aquaria Micro flow battery should be charged prior to use.
Stand by: BATT CHARGING is displayed. Display will read as Battery 100% when fully charged

- Remove lid from Aquaria Microflow
- Wipe the perforated lid and metal tabs that are exposed with an alcohol wipe
- Select a TSA or a Sabouraud media plate depending on whether fungal or bacterial monitoring is required
- Place the plate between the metal tabs. This will secure the plate into position. Replace lid
- Place the air sampler at designated position within the area to be monitored
- Switch on the Aquaria Micro flow

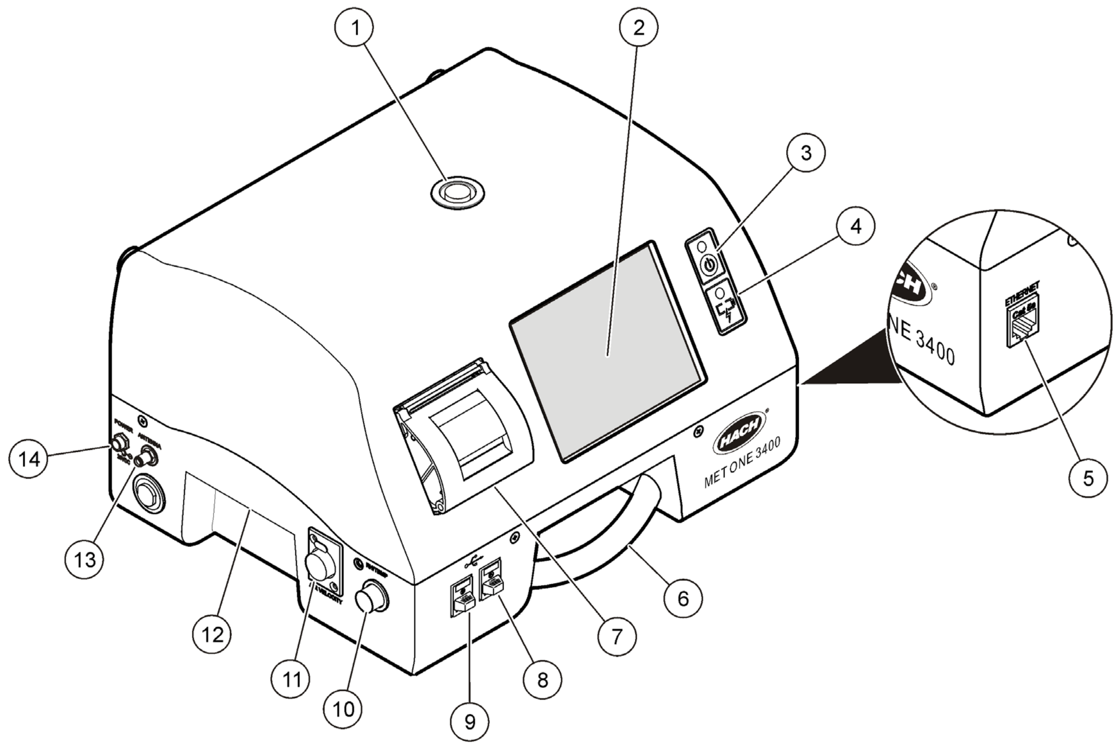
The Aquaria Micro flow is pre- set programmed to monitor 500 litres of air for 4 minutes 10 seconds at a flow rate of 120 L/min

To start the Aquaria Micro flow Press

- The screen will display Program sampling and a Time Countdown begins decreasing from 4 minutes 10 secs to Time 0 (finish)
- An alarm sounds when the time is complete and a RED light flashes
- Press ENTER/STOP
- Remove culture plate from Aquaria Microflow- the agar should be depressed with 'tram lines'
- Start process again for additional culture plates, wiping the perforated lid and metal tabs in between each sample
- If Programme requires to be altered - Press Prog
    - Select Programmed change, manual change or Sequential change by pressing up/ down arrows
    - Press Enter to select programme if required to change Volume of Air to be monitored, Flow Rate and Delay start
    - On completion of sampling, switch Air Sampler off
    - Return to lab, ensure Sampler is recharged before its next use and incubate plates according to their requirements, which are detailed in the relevant sections below

Particle Counting

The MET ONE 3400 is operated by use of a TFT colour touch screen located on the front panel of the unit. All commands are executed through the touch screen.



1 Sample intake nozzle
2 Touch screen
3 Power button
4 Battery status indicator
5 Ethernet connector
6 Handle
7 Printer
8 USB host connector
9 USB client connector
10 Relative humidity and temperature probe connector
11 Air velocity probe connector
12 Handle
13 Wireless antenna connector
14 Power connector

Particle counter navigation
The functions of the particle counter are accessed from the Counter Navigation screen

Icon	Function	Description
	Sample	Measures particle counts
	Historical	Review measurement results in the Buffer, print

Export — Output file as comma separated value (CSV), Tab separated, or PortAll files. Refer to the CD for information

. Printer — Print sample data as hard copy

Locations — Add/Edit/remove areas, copy location

Settings edit location settings, edit alarms for Specific locations.

Group — Load/add/edit a group, delete a group.

System — Time/Date. Sleep time/backlight timeout, set Logon requirements, set sounds for alarms, Manage users, set units for flow rates, and Manage the data buffer.

Sizes — Add/edit/delete a size (optional).

Test Wizard — Test and report wizard for ISO, EU-GMP, FS or BS classification compliance.

Return — Return to the previous screen or menu.

The Particle Counter battery should be charged prior to use.

- Batteries in the 3400 will begin to charge when the AC power cord is connected
- A complete charge in the instrument takes approximately 10 hours
- The battery is considered to be fully charged when they display shows the charge between 95% and 100%

Battery LED colour indications:

Flashing Orange/Low power = Not charging
Flashing Green/ Low power = Charging
Solid Green = Charged

To Log onto Particle Counter

- Remove the RED protective cap from the intake nozzle on the counter
- Start the system
- Locations do not need to be input as long as it is recorded onto the paper printout clearly

To Run a Particle Count

- To start the particle count, press the RUN (Green Diamond) button on the Counter Navigation screen
- This RUN button will change to a STOP (Red Octagon with a cross in the middle) button while the count is measured
- The Particle counter is set to perform 3 x 1 minute sampling and a Cumulative average is calculated and printed
- When the count measurement is complete, the test will stop automatically
- The results are automatically printed off
- This print out is returned to the lab from where the results are reported
- Print out is kept with the relevant daily worksheet
- The Cumulative average at 0.5µm is the figure routinely reported unless otherwise instructed
- After a complete particle count measurement, the number of particles measured will show on the screen and be stored as data. Other configured parameters, such as relative humidity, temperature and air velocity will be shown and stored in data
- On completion of sampling, switch counter off (Button3, counter diagram page 6)
- Ensure the sample intake nozzle is covered securely with a stopper once monitoring is complete
- Return to lab, ensure Sampler is recharged before its next use

7.2. Sampling Protocol

2 x BMS or BMS and a CSW will be required to go off site to monitor areas within Greater Glasgow and Clyde (GG&C) out with the GRI location.
Taxi transfer should be used to go to another location within GG&C. Authorisation information as regards ordering a taxi can be obtained from the Technical Manager or Operational Manager.

Weekly Monitoring:
The following sites require settle plates only
1. Environmental Lab, Microbiology Dept GRI
2. Mould Room, Microbiology Dept GRI

Monthly Monitoring:
The following sites requires a combination of contact plates, air sampling and particle counts performed
1. Gartnavel General Hospital Haematology (GGH Haematology Stem Cell Laboratory)
2. Bone Marrow Transplant Unit B8, B9 at Beatson Gartnavel General
3. Royal Hospital for Children, Ward 2A, Paediatric BMT Rooms
4. Nuclear Medicine, GRI

Quarterly Monitoring:
The following site requires a combination of air sampling and particle counts performed
1. Assisted Conception Suite (ACS) at GRI

Ad Hoc Monitoring:
Other areas which may be monitored on an ad hoc basis at the request of the Infection Control Consultant are
Theatres
Any other sites under GG&C

ICC may inform the laboratory of a new temporary testing regime or locations Along with this they will provide an email or a memo detailing the requirements. Each memo should include the following:
- Date of issue of memo
- Date of testing to commence
- Date of completion of testing (if known)
- Full testing requirements
- Reporting criteria

A copy of this will be put on the environmental notice board, within each copy of the SOP and staff will be informed at the All Staff Meeting.
If during the time frame of the ad hoc testing running any testing or reporting criteria changes, the memo must be updated and each copy replaced.
Once these ad hoc testing protocols conclude the memo should be removed for filing and staff informed that it is discontinued.

7.3. Site Specific Processing

A. <u>Environmental Lab, Microbiology Dept, GRI</u>

Refer to LP534 & record in LF521

B. <u>Mould Room, Microbiology Dept, GRI</u>

Refer to LP534 & record in LF521

C. <u>GGH General Hospital Haematology</u>

Address: 21 Shelley Rd, Level 2, Stem Cell Lab, Haematology, Gartnavel Hospital

On arrival at GGH Haematology to carry out Environmental Monitoring, access to Haematology dept is by Controlled door entry. Press buzzer for access. Staff at GGH should always know to expect you by prior arrangement.
The Stem Cell Processing lab is the only area monitored within Haematology.

<u>Particle Counts:</u>
- Test cabinet MC5177
- Test cabinet MC5176
- Bench (air in room)
- Outside air

Air Samples:

- Test BOTH cabinets
- Bench

Each Air Sample site requires:

- 2 x Sabouraud, incubate 1 at 22°C and 1 at 30°C, both for 7 days
- 1 x TSA, incubate at 30 °C for 7 days

Contact plates:

- Sample Sites are the work surfaces in BOTH cabinets
- Bench

Each contact plate sample site requires:

- 2 x Sabouraud, incubate 1 at 22°C and 1 at 30°C, both for 7 days
- 1 x TSA, incubate at 30 °C for 7 days

Received Plates:

Settle plates/Contact plates received from SCP are incubated as specified on their request form. (LF531)

Baxter's Hematron Bag Sealer:

Bag sealers are located at the side of each cabinet identified as Baxter's Hematron #1 and #2. They can be switched on at the back.
Using a sterile Trans swab, swab top and bottom of the 'sealing point' of each Hematron

- Before heating
- After heating (press blue button to heat)

Inoculate for each Hematron and for each before and after sample:

- CBA, incubate at 35°C for **48hr**
- Sabouraud, incubate at 30°C for 7 days
- TSA, incubate at 30°C for 7 days

D. Bone Marrow Transplant Unit (B8, B9) at Beatson Gartnavel General

Monitoring of the Beatson B8, B9 Unit requires prior arrangement before arriving at the Unit. Staff from Microbiology MUST present themselves to Beatson staff at the Unit upon arrival.

All staff should be aware that :

- laboratory coats are not to be worn for work carried out in Bone Marrow Transplant Unit (BMTU)
- A new plastic apron is to be worn on entering each room
- Alcohol gel to be used on hands on entering ward and each room

Particle Counts:

- Eight rooms are tested at random in each area of the 4th floor

(i.e. four rooms from B8 and four from B9) Please check with nursing staff before entering a room

- Corridor in each area (This provides a comparison with the count from the rooms sampled)
- Outside Air

Document if there is a lot of activity in the area at the time of monitoring

Air Samples:

- Eight rooms are tested at random in the B8/B9 Unit (the same rooms as for the Particle Counts)

Each sample site requires:

- 2 x Sabouraud plates
- incubate one at 22°C and one at 30 °C, both for 7 days

E. Royal Hospital for Children, Ward 2A

Monitoring of the paediatric BMT rooms (17-25) situated in ward 2A requires prior arrangement before arriving at the unit. Staff from microbiology must present themselves to ward 2A staff on arrival.

All staff should be aware that:

- laboratory coats are not to be worn for work carried out in Ward 2A
- A new plastic apron is to be worn on entering each room
- Alcohol gel to be used on hands on entering ward and each room

Particle counts:

- Four BMT rooms are tested at random - please check with nursing staff before entering a room
- Corridor
- Outside air

Document if any patients/others are in the room or if cleaners are in the area etc.

Air samples:

- The four BMT rooms in which particle counts have taken place

Each room will require:

- 1 x Sabouraud, incubate at 22°C for 7 days
- 1 x Sabouraud, incubate at 30°C for 7 days

F. Nuclear Medicine, GRI

Contact: Douglas Wright ████
Cabinet type: Amercare A2 Blood Cell Labelling Isolator
For cabinet layout refer to Appendix 1

Particle Counts:

- Inside Transfer Chamber
- Inside Main Cabinet
- Bench Area

Each of the enclosures, Main Cabinet and Transfer Chamber, are fitted with a sample point. This has a removable bung through which the particle count probe can be fed.
The sample point for the main cabinet is situated on the top of the cabinet and the point for the transfer chamber on the front: top left side of the door.
NB. For main cabinet sampling, remove the tripod from large particle sampling tubing and attach the thinner sampling tubing. Push this thinner tubing into place through the top port. Wait approx. 2 minutes before sampling to allow air to settle.
(To remove the white bung simply rotate the top by half a turn anti clockwise. The particle count tube can then be passed through)

Air Samples:

- Inside Transfer chamber
- Inside Main Cabinet
- Bench Area

Each area will requires:

- 1 x SAB, incubate 22°C for 7 Days
- 1 x SAB, incubate 30°C for 7 Days
- 1 x TSA, incubate 30°C for 7 Days

Within the cabinet chambers the air samplers require a two minute delay. This ensures that the air pressure has stabilised.
NB. Before the cabinet inner door can be opened, there is a time delay, preset, the end of which is noted by a beep.
Outer door switch, open door, load samplers, replace door, press door control to seal, two min delay, beep, press inner door foot pedal and remove inner door.
To recreate vacuum on exit press foot pedal

Received Plates:

All Settle plates received from Nuclear Medicine GRI are incubated at 30°C for 7 days

G. Assisted Conception Suite at GRI (ACS)

Monitoring of the Assisted Conception Suite requires prior arrangement before arriving at the unit.
Microbiology staff will be escorted to the staff changing lockers by ACS staff where they will be requested to change into scrubs.
All staff is to be aware that this is a highly sensitive area, staff for Microbiology should always check with ACS that it is appropriate at that particular time to enter certain areas, namely procedure rooms, as work with a patient may be ongoing.
ACS staff will direct Microbiology staff to the Main Lab for testing to begin.

Please refer to Appendix 2: ACS Floor plan for highlighted areas requiring testing:

Main Lab Area			
1-Cabinet 1	2-Cabinet 2	3-Cabinet 3	4-Cabinet 4
5-Cabinet 5	6-Cabinet 6	10-Main lab area left	14-Main lab area right
Andrology Lab			
7-Cabinet 7	8-Cabinet 8	19-Area within Andrology Lab	
Sterile Procedure Room			
25-Scrub Area	28-Bench area		
Procedure Room 2			
33-Corner of room			
Lobby			
23-Area opposite sink			

Particle Counts:

- Each site mentioned above
- Corridor outside the Lab

Air Samples:

- Same sites as Particle counts except the corridor outside the lab does not require air sampling

Each site will require:

- One Sabouraud, incubate 30°C for 7 days
- One Sabouraud, incubate 22°C for 7 days
- One TSA, incubate 30°C for 7 days

Received Plates:

On occasion, to monitor ACS staff competency in their Aseptic Technique, Contact plates and TSA agar plates may be requested from the unit.
On receipt back in the lab these are to be incubated 30°C for 7 days and a final report issued with culture result.

H. Theatres

There is sufficient evidence to support the undertaking of microbiological air sampling:

1. As part of the commissioning of an operating theatre
2. After any major restructuring refurbishment including alterations to the fabric of the theatre or changes to the ductwork distribution that may affect airflow to or within the theatre suite. (Minor changes to ductwork may not necessitate sampling – discuss with Infection Control)
3. As deemed necessary by Infection Control e.g. as part of an outbreak investigation

Procedure for Theatre Air Samples

- The theatre should have had an in-depth clean and be thoroughly clean and dust free at least one hour before sampling

- **It is crucial that no- one should have entered the theatre in the one hour prior to air sampling**

- The air handling unit should have been operating at normal flow rates continuously for at least 24hours before sampling in a new theatre and for one hour in an existing theatre
- The air sampler should be cleaned before use with 10% Distel on the outside and alcohol wipe on the inner plate and metal tabs
- It should run briefly before the agar plate is loaded to blow any contamination out of the sampler
- Air Sampling is carried out in 2 positions using TSA plates with a sample size of 500L. Set time delay at 15 minutes to allow enough time to exit room and air flow to settle
- Incubate **TSA at 37° C for 2 days**

The results from the two TSA plates used for air sampling are **added together.** This will give a **final count of cfu/m^3**

HTM 2025 Commissioning of Theatres gives a pass at less than 10 colony forming units in one cubic metre of air

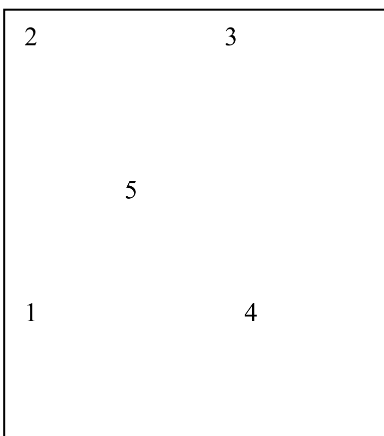
- **PLEASE INFORM INFECTION CONTROL CONSULTANT IF RESULT IS > 10 cfu/m^3**

There may be, on occasion, a request for samples for fungal culture. Two Sabouraud plates should be used at each site at which the TSA samples were taken. Incubate, one SAB from each site: 30°C 7days
: 22°C 7days

Procedure for Particle Counts (Orthopaedic Theatres Only)

- Prior arrangements are made with theatre sister to make sure air flow cabinet is left running. Alternatively switch on to operational speed
- Prior to entering theatre area, theatre greens, overshoes and head covering must be worn
- Each theatre is tested at 5 specified sites underneath micro flow cover
- Each test location should be numbered 1-5 , and recorded on the print out

(Doors)



- Printout is inspected and noted as a pass or fail
- Result interpretation- each theatres' particle count should be < 100 particles at 0.5 µm

7.4. Booking in Procedure

For laboratory taken samples complete a request form & daily worksheet, recording date of sampling

For settle plates arriving at laboratory, ensure the sample details match the request form, record date & time of receipt of the samples

For both ensure sufficient information is recorded on the request form to issue a report (i.e. comments required)

- Label samples using designated Environmental laboratory numbers
- Ensure each sample/site is given an individual lab. number
- Record lab. numbers on Air Sampling day sheet (LF525)
- Incubate the culture plates accordingly
- Particle (Cumulative Average at 0.5 µm) and Culture results are recorded onto the Air Sampling day sheet, transcribed onto the request form then reported

7.5. Examination of Plates

<u>Reading Plates</u>

TSA
Examine plates for growth of bacteria & fungus

- Perform a total bacterial count and record on daily worksheet
- Perform a total fungus count and record on worksheet

- Identify all fungus isolated

SAB
Examine plates for growth
- Perform a total count and record on worksheet

- Identify all fungus isolated

BA

Examine plates for growth of bacteria & fungus

- Perform a total bacterial count and record on worksheet
- Perform a total fungus count and record on worksheet

- Identify all fungus isolated

Identification of Growth

Bacterial

- No identification required routinely

Fungus

- Complete Environmental/Mycology ID Request form, which includes Lab no. & Sample Location
- Pass plates and photocopied form to Mycology for identification
- Mycology will assign new number and PID and result enter

7.6. Reporting Results

Bacterial Counts (Air Samples/Contact/Settle Plate)

- Copy bacterial count result to request form
- Sign and date
- Pass completed form to office
- Office staff photocopy report, send PDF via generic email and post photocopy
- Original form filed

Fungal Counts (Air Samples/Contact/Settle Plate)

- Copy fungal count result to request form
- Sign and date and record as interim

Interim results:

Issue report with fungal count awaiting identification

- Pass completed request forms to office to issue interim report
- Office staff photocopy report, send PDF via generic email. Original request form returned to environmental lab and placed in Interim folder

Final results:

Issue once mycology result received (blue hard copy)

- Photocopying blue form and match to worksheet (file in lab for 1 month, after this pass to office for filing)
- Attach blue copy to request form and record as final, sign and date
- Pass completed request forms to office to issue final report
- Office staff photocopy report, send PDF via generic email. Original form stored

Particle Counts

- Particle printout attached to worksheet
- Copy particle count result to request form & worksheet
- Sign and date

- Pass completed request forms to office to issue final report
- Office staff photocopy report, send PDF via generic email. Original form stored in filing
- Worksheet filed in lab for 1 month, after this pass to office for filing

7.7. Notification of Results

Air Samples:

For all areas sampled, except Stem Cell lab, report any growth of fungus to Infection Control Consultant.

For stem cell lab – report any growth of fungus to stem cell lab Quality Manager (QM) and Technical Manager (TM)

Operating theatres >10cfu/m³ - report to Infection Control Consultant.

Particle Counts:

A. Assisted Conception Suite and Stem Cell Processing Lab

Acceptable counts are listed below. Any counts out with these ranges report to Infection Control Consultant for ACS and QM/TM for stem cell lab.

Grade	At rest particle count		In operation particle count	
	≤0.5 µm	5 µm	≤0.5 µm	5 µm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Undefined	Undefined

B. Beatson B8/9/RHC Ward 2A

> 1000 particles at 0.5um in any room – report to Infection Control Consultant

C. Operating theatres

> 100 particles at 0.5um – report to Infection Control Consultant

Contact Plates:

A. Assisted Conception Unit/Stem cell lab

Acceptable results below – out with these contact Infection control consultant for ACS and QM/TM for stem cell lab

Grade	Settle plates (90mm diameter) cfu/plate	Contact plates (55mm diameter) cfu/plate
A	< 1	< 1
B	5	5
C	50	25
D	100	50

B. GRI Lab Environmental Settle Plates:

All plates from GRI labs have results recorded on LF534. No identification or result entry required. Refer to LP534 for full information

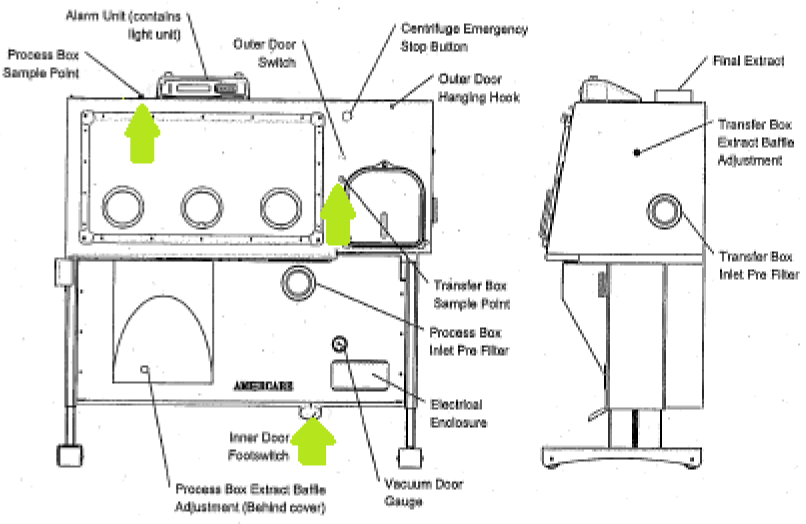
8. References

SOP 042 Assisted Conception Service
SHTM 0301
SOP BMT 100 057 01 Stem Cell Transplantation service SOP

Appendix 1: GRI Nuclear Medicine

Section 7 - Drawings

7.1 Main Layout Drawing



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	Authorised by: Sandra Higgins
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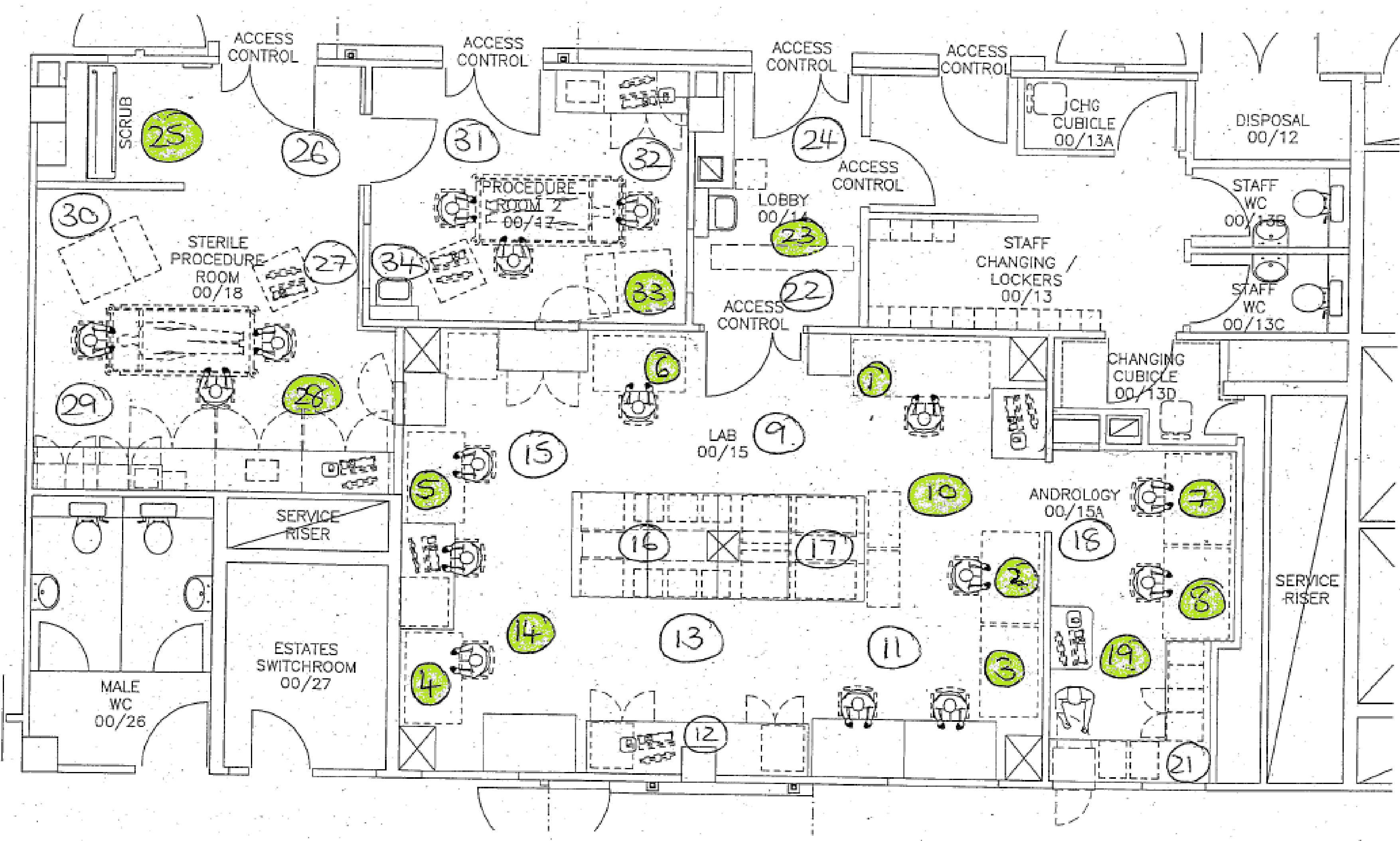
Appendix 2 : Assisted Conception Sampling Sites



SCOTTISH HOSPITALS INQUIRY
Bundle of documents for Oral hearings commencing from 19 August 2024 in relation to the Queen Elizabeth University Hospital and the Royal Hospital for Children, Glasgow
Bundle 27 – Volume 15 – Miscellaneous Documents