**Scottish Hospitals Inquiry**

**Post Oral Evidence Statement**

**Dr Teresa Inkster**

In relation to 'picks' and the sequencing report prepared by Professor Leanord and Derek Brown dated 18 January 2023, which can be found at **Bundle 6, page 1195**, in the course of carrying out a DNA analysis, particularly by whole genome sequencing, one or more colonies or 'picks' will be selected for that process.

1. Q. Are you aware of any published material which discusses the number of such 'picks' which should be used to ensure reliable results or conclusions? If so, please identify any such publications, and if possible, attach copies or links to your reply.

A. I am not aware of any publica on that concludes exactly how many picks are enough to ensure reliable results or conclusions. I am however, aware of several publications which discuss the limitations of selecting a single colony pick including examples where greater than one colony pick has been undertaken. These are listed in the table below along with the relevant parts of the paper and copies are a ached. I would like to highlight that the issue with colony picks was discussed with Professor Leanord in January 2021. Dr Michael Weinbren, Consultant Microbiologist and Clinical Lead for water at the me with NHS Assure, requested changes to the research application for Cupriavidus WGS to include multiple picks from water samples. This was acknowledged by Professor Leanord and included in the research application as a secondary question. The application states 'Current national guidance asks laboratories to select a single colony from an agar plate for typing of positive water samples. This approach may underestimate exogenous acquisition of waterborne pathogens. Outbreaks may be polyclonal, particularly if mature and extensive biofilm is present in pipework/outlets. Not detecting a polyclonal outbreak is a lost opportunity for future preventative measures .' Emails and the application form have been provided to the Inquiry. ( Cupriavidus CoE WGS proposal 221220 Final v3 Al edits (**Bundle 27 Volume 17 document 35**) Email chain between M Weinbren and A Leanord regarding WGS protect 17-19 January

2021 **(bundle 27 volume 17 document 36)**

| Publication | Relevant sections |
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| Vallés J, Mariscal D, Cortés P, Coll P, Villagrá A, Díaz E, Ar gas A, Rello J. Pa erns of colonizationon by Pseudomonas aeruginosa in intubated pa ents: a 3year prospective study of 1,607 isolates using pulsed-field gel electrophoresis with implications for prevention of ventilator-associated pneumonia.<br>Intensive<br>Care Med. 2004 Sep;30(9):1768-75<br>**A50508336** | In the discussion sec on the authors state 'This study is also unique in subculturing at least four colonies that were representative of the different morphological types of PA present on each culture plate. It allowed the origin of the strains to be identified and reduced the risk of underestimating exogenous colonization' |
| Visca P, Goldoni P, Lück PC, Helbig JH, Ca ani L, Giltri G, Brama S, Castellani Pastoris M. Multiple types of Legionella pneumophila serogroup 6 in a hospital heated-water system associated with sporadic infections. J<br>Clin<br>Microbiol. 1999 Jul;37(7):2189-96 | In the discussion sec on the authors state 'Second, sampling bias can occur, and a large number of environmental isolates should be genotyped to ensure that all types of legionellae present in the sample are recovered and characterized.' |

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| Mäklin T, Kallonen T, Alanko J, Samuelsen Ø,<br>Hegstad K, Mäkinen V, Corander J, Heinz E, Honkela A. Bacterial genomic epidemiology with mixed samples. Microb Genom. 2021 Nov;7(11):000691.<br>**A50808339** | Introduction sec on states 'Standard genome based epidemiological linking of cases requires accurate genome sequences for the pathogens derived from high coverage sequencing data for pure-colony isolates. The isolates are obtained by an enrichment and separation step in the form of a plate culture and subsequent colony picks based e.g. on morphology and colour. Typical workflow of genomic epidemiology may thus necessitate multiple colony picks per sample with the corresponding DNA library preparation and sequencing steps done individually for each of them. DNA isolation, library prep and sequencing require a significant amount of laboratory effort and me per colony, and lead to increased costs since the price of library preparation is becoming comparable to the cost of sequencing itself.' |
| Stoesser N, George R, Aiken Z, Phan HTT,<br>Lipworth S, Quan TP, Mathers AJ, De Maio N,<br>Seale AC, Eyre DW, Vaughan A, Swann J, Peto<br>TEA, Crook DW, Cawthorne J, Dodgson A, Walker AS; TRACE Investigators Group. Genomic epidemiology and longitudinal sampling of ward wastewater environments and pa ents reveals complexity of the transmission dynamics of bla$_{KPC}$-carbapenemaseproducing<br><br>**A50808334** | See diagrams page 8 Figure 5 for the number of colony picks taken. This paper in general highlights the complexities of WGS in wastewater environments. |

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| Enterobacterales in a hospital se ng. JAC Antimicrob Resist. 2024 Sep 3;6(5):dlae140 | |
| Tang P, Croxen MA, Hasan MR, Hsiao WWL, Hoang LM. Infection control in the new age of genomic epidemiology. Am J Infect Control. 2017 Feb 1;45(2):170-179.<br><br>**A50803442** | Discussion sec on 'Tracking transmission dynamics, however, can be hampered by the diversity of organisms that can be found within a single host. Sequencing of multiple colonies of the same organism isolated from a single person showed that these individuals can carry multiple unrelated types of that species, such as diverse sequence types of MRSA, extendedspectrum β-lactamase–producing E coli or vancomycin-resistant enterococci. SNVs within a single sequence type have been seen in MRSA, A baumannii, and K pneumoniae, and this has been described as a cloud of diversity. These findings imply that a single colony isolated from a single patient may be insufficient to reconstruct the chain of transmission between otherwise epidemiologically linked patients. This diversity is not exclusive to infected patients; asymptomatic carriers can also harbor a diverse set of potential pathogens that can be silently introduced into health care facilities and linked to outbreaks. These organisms may have a unique biology that makes them adept at establishing a foothold in health care settings.' |

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| Van Goethem N, Descamps T, Devleesschauwer B, Roosens NHC, Boon NAM, Van Oyen H, Robert A. Status and potential of bacterial genomics for public health practice: a scoping review. Implement Sci. 2019 Aug 13;14(1):79<br><br>**A50803448** | Discussion sections; The most highlighted issue is the fact that WGS, as is equally the case for conventional typing methods, cannot stand on its own and that epidemiological data (including me, place, and exposure data) should complement the WGS results to identify a common source or link cases during outbreak investigations. False conclusions could be drawn from WGS data alone since it is possible that epidemiologically unrelated isolates are highly similar at the SNP level. Another reported issue was the potential misinterpretation of isolate relationships given the diversity of isolates that can be found within a single host (e.g., following long term carriage) or environmental reservoir. It was stressed by several studies that it is important to account |
| | for this “cloud of diversity” by increasing the number of samples taken from the suspected source. |

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| Jay-Russell MT, Mandrell RE, Yuan J, Bates A,<br>Manalac R, Mohle-Boetani J, Kimura A, Lidgard J, Miller WG. Using major outer membrane protein typing as an epidemiological tool to investigate outbreaks caused by milk-borne Campylobacter jejuni isolates in California. J Clin Microbiol. 2013 Jan;51(1):195-201.<br><br>**A58088338** | Methods sec on; Multiple (8 to 12) pure colonies of presumptive C. jejuni were picked from each plate to increase the likelihood of finding the clinical outbreak subtype and stored on Microbank dry beads<br>Discussion: Our analysis also emphasizes the importance of selecting multiple pure colonies from individual samples during C. jejuni outbreak investigations for successful molecular subtyping, as described previously in the human and veterinary literature |

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| Ruppé E, Olearo F, Pires D, Baud D, Renzi G,<br>Cherkaoui A, Goldenberger D, Hu ner A, François P, Harbarth S, Schrenzel J. Clonal or not clonal? Investigating hospital outbreaks of KPCproducing Klebsiella pneumoniae with whole genome sequencing. Clin Microbiol Infect. 2017 Jul;23(7):470-475.<br><br>**A58088343** | Discussion; Perhaps if we had considered other sites for sampling and selected more than one strain from the culture, we might have identified the strain that had been transmitted to HUG2 and HUG3. Currently no specific guidelines recommend the typing of more than one strain per patient. These findings suggest that when typing with WGS, the collection of multiple strains from more than one anatomic site could be justified.’ |

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| Quick J, Cumley N, Wearn CM, Niebel M,<br>Constantidou C, Thomas CM, Pallen MJ,<br>Moiemen NS, Bamford A, Oppenheim B, Loman NJ. Seeking the source of Pseudomonas aeruginosa infections in a recently opened hospital: an observational study using whole genome sequencing. BMJ Open. 2014 Nov 4;4(11):e006278.<br><br>**A50808334** | Method; For storage and DNA extract on a single colony was purified from the primary culture plate. When different colony morphologies were observed, a single colony from each type was purified. Additionally, for a randomly selected water sample, 24 colonies were individually picked from one waterfilter primary microbiological plate for sequencing.<br><br>Results; A total of 86 genome sequences were generated from the 71 positives, as in some cases multiple colony picks were sequenced. Seventyeight patient samples were screened for P. aeruginosa of which 39 (50%) were positive. A total of 55genome sequences |
| | were generated, as in some cases multiple colony picks were sequenced. |
| Environment agency – Methods for the examination of waters and associated materials Archived 2018<br>**A50808335** | Sec on 8.3.3 and 9.7.1 |
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I believe that the facts stated in this witness statement are true. I understand that proceedings for contempt of court may be brought against anyone who makes, or causes to be made, a false statement in a document verified by a statement of truth without an honest belief in its truth.

The witness was provided access to the following Scottish Hospital Inquiry bundles/documents for reference when they completed their questionnaire/ statement (Appendix A)

The witness verbally introduced or provided the following documents to the Scottish Hospital Inquiry for reference when they completed their questionnaire statement (Appendix B)

**Appendix A**

**A50071192 -** Bacteraemia rates and Resistance Paediatric Haemato-oncology 20142018 **(Bundle 27, Volume 6, page 107)**

**A50071192** - Environmental Organisms, Antibiotic use and Antibiotic Resistance – **(Bundle 27, Volume 6, page 121)**

**A42401483** Report- Application of Whole Genome Sequencing Alastair Leanord and Derek Brown **(Bundle 6 page 1195)**

**Appendix B**

**A50808335** Cupriavidus CoE WGS proposal 221220 Final v3 Al edits (**Bundle 27 Volume 17 document 35)**

**A50808337** Email chain between M Weinbren and A Leanord regarding WGS protect 17-19 January 2021 **(bundle 27 volume 17 document 36)**

**A50808336** Patterns of colonisation of Pseudomonas aeruginosa: a 3-year prospective study Intensive Care Medicine **(Bundle 27 Volume 17 document 37** Multiple types of Legionella pneumophila serogroup 6 in a hospital heated-water system associated with sporadic infections **(Bundle 27 Volume 17 document 34)**

**A50808339** Bacterial genomic epidemiology with mixed samples. Microb Genom. 2021 (**Bundle 27 Volume17 document 38)**

**A50808334** Genomic epidemiology and longitudinal sampling of ward wastewater environments and patients reveals complexity of the transmission dynamics of blaKPC-carbapenemase-producing Enterobacterales in a hospital se□ng. JAC Antimicrob Resist. **2024 (Bundle 27 Volume 17 document 39)**

**A50808342** Infection control in the new age of genomic epidemiology. Am J Infect

Control. (**Bundle 27 Volume 17 document 40)**

**A58088341** Using major outer membrane protein typing as an epidemiological tool to investigate outbreaks caused by milk-borne Campylobacter jejuni isolates in California. J Clin Microbiol. (**Bundle 27 Volume17 document 41)**

**A50808343** Clonal or not clonal? Investigating hospital outbreaks of KPCproducing Klebsiella pneumoniae with whole-genome sequencing. Clin Microbiol Infect. **(Bundle 27 Volume 17 document 42)**

**A5088344** Seeking the source of Pseudomonas aeruginosa infections in a recently opened hospital: an observational study using whole-genome sequencing. (**bundle 27 Volume 17 document 43)**

**A50883345** Environment agency – Methods for the examination of waters and associated materials (**Bundle 27 Volume 17 document 44)**