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O0858 Metrological characterization of the reproducibility of MALDI-TOF MS for bacterial strain typing

Eloise Busby*[1 2], Jim F Huggett[1 3], T.D. Mchugh[2], Emmanuel Wey[2 4]

[1]LGC, Molecular and Cell Biology, Teddington, United Kingdom, *[2]UCL, Centre for Clinical Microbiology, London, United Kingdom*, *[3]University of Surrey, School of Biosciences & Medicine, Guildford, United Kingdom*, *[4]Royal Free London NHS Trust, London, United Kingdom*

Background: There are limited studies characterising the reproducibility of matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) for bacterial strain typing. This could prevent the use of MALDI-TOF MS as a reliable alternative to current genomic techniques. We challenged aspects of the Bruker MALDI Biotyper protocol to identify sources of error and assess their impact on proteomic characterisation of antimicrobial resistant bacteria.

Materials/methods: *Klebsiella pneumoniae* protein extracts were obtained at various time points: 1) 24 hours following recovery from cryo-storage, 2) over a period of 72 hours with sub-culture every 24 hours and 3) over 24, 48 and 72 hours without subculture. Extracts were also obtained at 24 hours following recovery for additional bacterial species at baseline (T0), and resampled one year later (T1). Spectra were acquired on a MALDI-TOF Microflex (Bruker UK) and analysed in FlexAnalysis (3.4). Additionally, spectra were exported for statistical analysis in BioNumerics 7.6 (Applied Maths).

Results: Spectral acquisition from the same extract was highly repeatable, with replicate peaks falling within 0.025% of the m/z value assigned in FlexAnalysis. Peak classes between conditions 1) and 2) were concordant, however peak shifts were observed between 24 and 48 or 72 hours in condition 3). BioNumerics Mann-Witney U test revealed that up to 80% of assigned peak classes were significantly different ($p < 0.05$) between extracts in condition 3). Furthermore, statistically significant differences were observed between spectra obtained at T0 and T1 across a range of species. While strain typing could be inconclusive at different time points, species identification remained unaffected for all samples tested in this study.

Conclusions: The discordance in peak classes in condition 3) may reflect senescence-related proteomic changes that can occur in culture. Cryopreservation of isolates may also lead to significant differences between spectra. Although species identification remained unaffected, supporting the robustness of MALDI-TOF MS for this purpose, protein profiles may be influenced by culture or long-term storage. While the repeatability of MALDI-TOF MS enables consistent peak calling that may be used for strain typing, our findings suggest that further work is required to standardise the upstream culture stages of the protocol.