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Multicentre study on the improved identification of non-tuberculous mycobacteria with MALDI-TOF MS using three different preprocessing protocols

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Background: The implementation of MALDI-TOF MS technology in clinical microbiology laboratories has allowed a rapid and accurate identification of Non-Tuberculous Mycobacteria (NTM) isolates. However, the rate of successful identifications highly depends on the staff experience and the processing of the samples prior to MALDI-TOF MS identification. In this project, 14 labs -with high expertise using MALDI-TOF MS- from 10 EU/EEA countries have tested different processing methods in order to evaluate their accuracy and efficiency.

Material/methods: Twelve NTM isolates were grown on Löwenstein-Jensen medium. Biomass was collected with a 1 ml sterile loop in tubes containing 300 μl water and 900 μl ethanol and sent out to the participating laboratories in triplicates. Each laboratory tested one of the triplicates using the regular protocol developed by the manufacturer (Bruker Daltonics, Bremen, Germany), one with an additional step of 1-minute bead-beating and the last one with a 15-minute sonication step. Samples were identified in triplicates using MALDI-TOF MS, with the MALDI Biotyper Mycobacteria Library v3.0 and v4.0. The first and second identifications with higher score from each sample were recorded for consistency reasons, as well as their score.

Results: The protocol that included the bead-beating step provided a rate of 89.7% correct identifications, with 2 isolates misidentified in one laboratory and 14 that produced no peaks in 4 laboratories (3 each in two labs, 1 in another and 7 in the last one). Identifications obtained with this protocol were highly consistent, with high scores for *M. abscessus*, *M. kansasii* and *M. mucogenicum/phocaicum*. The second procedure –MycoEX protocol by Bruker Daltonics– yielded 97.0% correct identifications with average score values above 2.0 for *M. fortuitum*, *M. mucogenicum/phocaicum*, *M. kansasii*, *M. avium*, *M. triplex* and *M. gordonae*. Only one isolate was misidentified (a *M. kansasii* isolate identified as *M. gastri* as top identification and *M. kansasii* as second option) and 4 isolates produced no peaks in 3 labs. The third protocol evaluated –with a sonication step– allowed 98.0% correct identifications. No isolates were misidentified with this protocol and 3 isolates produced no peaks in 2 labs, one due to problems during transportation. Most of the species identified with this protocol obtained a score value close to or above 2.0.

Conclusions: Improvements in the preprocessing steps of NTM have allowed a more accurate and consistent identification of these microorganisms using MALDI-TOF MS. Between 89.7% and 98.0% of the isolates were reliably identified with the evaluated protocols. The sonication step allowed a higher rate of successful identifications. This would be the method of choice for laboratories where a

sonicator is available. However, the other two protocols would also yield a high rate of correct identifications of NTM isolates from subcultures.