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MALDI-TOF: driving change in microbiology laboratories

MALDI-TOF MS identification of non-tuberculous mycobacteria directly from solid-medium colonies using a simplified method

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Background: Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has recently proved its effectiveness identifying Non-Tuberculous Mycobacteria (NTM). However, sample preparation prior to MALDI-TOF MS identification is time-consuming and difficult to integrate in the routine of a busy microbiology laboratory. It consists on a protein extraction with ethanol, several centrifugation steps and vortexing with silica beads and formic acid/acetonitrile. In this study we evaluated the performance of MALDI-TOF MS directly on colonies from NTMs grown on solid medium, using a simplified, on-plate extraction method using 100% formic acid plus a standard protein extraction step, only when required.

Material/methods: Fifty consecutive NTM isolates from clinical specimens received for routine identification with Genotype CM/AS (Hain Lifescience) were included in the study. They belonged to 8 rapid growing and 8 slow growing mycobacteria species. The simplified protocol had been presented in the ECCMID 2015 (Calvo-Reyes et al. Poster EP229). Briefly, colonies grown on Löwenstein-Jensen medium were collected in an eppendorf tube containing 300µl of HPLC-grade water and inactivated for 30 min at 95°C under Biosafety Level 3 conditions. Once centrifuged, the pellet was spotted directly on a stainless steel MALDI-TOF target plate and covered with 1 µl of 100% formic acid. Once dried, 1 µl of matrix (α-cyano-4-hydroxycinnamic acid solution in 50% acetonitrile and 2.5% trifluoroaceticacid) was added. When no identification could be obtained with this simplified method, a standard in-tube protein extraction using 70% formic acid and the same amount of 100% acetonitrile was applied. NTM isolates were analysed by MALDI-TOF MS, using a MicroflexLT benchtop mass spectrometer (Bruker Daltonics, Bremen,Germany).

Results: All the rapid growing mycobacteria analyzed (22) were correctly identified by MALDI-TOF MS with the simplified method with score values≥1.8 except in two cases: one *M. fortuitum* and *M. porcinum* isolates identified with score values of 1.742 and 1.787, respectively.

Of the 28 slow growing mycobacteria isolates tested, 21 (75.0%) were reliably identified using the simplified method. Besides, 5 more isolates could be identified by performing the protein extraction step. Only 2 isolates (one *M. avium* and one *M. kansasii*) could not be identified using this procedure and required the protocol recommended by the manufacturer for a final identification.

In total, 100% of the rapid growing and 92.85% of the slow growing mycobacteria could be directly identified using MALDI-TOF MS.

Conclusions: The use of a simplified method for the identification of Non Tuberculous Mytcobacteria isolates with MALDI-TOF MS provides rapid and accurate results for all the rapid growing mycobacteria and most of the slow growing ones. Thus, our recommendation is to use the simplified method for all the NTM and apply the protocol developed by the manufacturer only for those NTM isolates with no reliable MALDI-TOF identification with the simplified method.