AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Fails To Identify Nontuberculous Mycobacteria from Primary Cultures of Respiratory Samples

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We have assessed matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification (Bruker) of nontuberculous mycobacteria from newly positive liquid cultures of respiratory samples. Twelve (22%) of 54 isolates were identified directly from liquid medium. After subculture and with manual laser operation, this rose to 49/54 isolates (91%). MALDI-TOF MS is less promising than previously suggested.

Nontuberculous mycobacteria (NTM) are increasingly recognized as mostly opportunistic pathogens of humans. The most frequent disease manifestation is a chronic pulmonary infection (1). Since NTM are environmental microorganisms, their presence in pulmonary samples need not indicate disease *per se*. The clinical relevance of NTM isolation differs strongly by species (2). Hence, correct identification is of paramount importance (3).

Identification of NTM is done mostly by using molecular tools. While accurate, these require a good laboratory infrastructure and trained personnel and are costly. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has revolutionized identification in general bacteriology and has also been tried for NTM (3). Most studies of MALDI-TOF MS identification of NTM have applied it to pure cultures from strain collections (4) rather than new positive cultures from clinical samples from nonsterile sites.

We assessed MALDI-TOF MS identification of NTM directly from new positive liquid cultures of respiratory samples in a mycobacterial disease reference clinic.

Primary cultures of respiratory samples were performed with the Mycobacterium Growth Indicator Tube (MGIT) system (BD Bioscience, Erembodegem, Belgium), as well as on Löwenstein-Jensen slants, both at 37°C, after decontamination by the 1% *N*-acetyl-L-cysteine-sodium hydroxide method (3).

All of the positive cultures that we obtained during the March-April 2014 and January-February 2015 periods that tested negative in the TBc-ID immunochromatography assay (BD Bioscience) were studied.

We performed MALDI-TOF MS identification with the MALDI Biotyper (Bruker Daltonics, Bremen, Germany) platform. Protein extraction from liquid MGIT medium for MALDI-TOF MS analysis was performed by the manufacturer's MycoEx protocol. In short, we collected biomass by aspirating 1.2 ml of liquid medium from the bottom of the MGIT tube and transferring it to a reaction tube, which was then centrifuged for 2 min at 13,000 rpm (8,124 × *g*; 43-mm rotor radius); the supernatant was pipetted off, the pellet was resuspended in 300 μl of high-performance liquid chromatography grade water, and cells were then inactivated by boiling for 30 min. A 900-μl volume of pure cold ethanol was then added, and the tube was vortexed and centrifuged for 2 min at 13,000 rpm. The supernatant was pipetted off. The last two steps were repeated once. The pellet was dried at room temperature before the addition of zirconium beads and 10 to 50 μl of pure acetonitrile, depending on the pellet size. After 1 min of vortexing, we added an identical volume of 70% formic acid and vortexed the mixture again. After centrifugation for 2 min at 13,000 rpm, 1 μl of the supernatant was spotted onto the target and, once dry, overlaid with 1 μl of hydroxycinnamic acid matrix.

Extraction was started within 24 to 48 h after the MGIT tube was flagged positive; tubes remained in the MGIT machine. After 1.2 ml was taken from the liquid medium for direct MALDI-TOF MS analysis, a cotton swab was used to sample the bottom of the MGIT tube and streaked onto a Middlebrook 7H11 plate (BD Bioscience, Erembodegem, Belgium). After 4 to 6 days of incubation, plates were swabbed with a cotton swab and biomass was transferred to a reaction tube with 300 μl of deionized water. From this, MycoEx extraction was then performed, starting with inactivation. The peak patterns obtained were compared to the Mycobacteria 2.0 database (Bruker Daltonics, Bremen, Germany), which does not distinguish between *Mycobacterium intracellulare* and *M. chimaera*.

With the material remaining in the MGIT tube, we performed molecular identification with the Inno-LiPA Mycobacteria v2 line probe assay (Innogenetics, Ghent, Belgium) supplemented by partial *hsp65* gene sequencing (5). This algorithm is insufficient to identify *M. abscessus* isolates to the subspecies level (6).

Fifty-four new positive cultures from respiratory specimens were subjected to identification. The molecular test identified the mycobacteria as *M. avium* ($n = 23$), *M. chimaera* ($n = 11$), *M. intracellulare* ($n = 4$), *M. abscessus* ($n = 4$), *M. gordonae* ($n = 4$), *M. chelonae* ($n = 2$), *M. kansasii* type 1 ($n = 2$), *M. fortuitum*, *M.*

Received 10 February 2016 **Returned for modification** 4 March 2016
Accepted 29 April 2016

Accepted manuscript posted online 4 May 2016

Citation van Eck K, Faro D, Wattenberg M, de Jong A, Kuipers S, van Ingen J. 2016. Matrix-assisted laser desorption ionization–time of flight mass spectrometry fails to identify nontuberculous mycobacteria from primary cultures of respiratory samples. J Clin Microbiol 54:1915–1917. doi:10.1128/JCM.00304-16.

Editor: A. J. McAdam, Boston Children's Hospital

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TABLE 1 MALDI-TOF MS scores obtained directly and after subculture

Method	No. of samples with which following MALDI-TOF MS score was obtained with:										
	Default machine settings only:					Manual laser operation:					
	>2.3	2.0–2.3	1.7–2.0	<1.7	NP[a]	>2.3	2.0–2.3	1.7–2.0	<1.7	NP	Total
MGIT	0	7	5	3	39	0	17	14	20[b]	3	54
7H11 subculture	4	20	5	4	21	6	33	10	5[c]	0	54

[a] NP, no peaks found.
[b] Nine wrong identifications.
[c] One wrong identification.

malmoense, and *M. lentiflavum* (all $n = 1$); there was 1 mixed culture of *M. avium* and *M. kansasii* type 1.

The MALDI-TOF MS scores are presented in Table 1; the manufacturer-recommended score threshold for definite species level identification (>2.3) was hardly ever achieved. Subculture on solid medium and manual laser operation were needed to get good-quality identification results for 49/54 (91%) of the isolated NTM. Only 7/54 isolates (13%) could be identified directly from the MGIT medium with identification scores of >2.0, and 12 (22%) with scores of >1.7 could be identified. With manual laser operation, this rose to 17/54 (31%) and 31/54 (57%). Subcultures analyzed with default settings led to only 29/54 (54%) isolates being identified with scores of >1.7. The scores obtained did not differ between extractions started after 24 or 48 h after the MGIT was flagged positive.

All identifications based on MALDI-TOF MS scores of >1.7 were concordant with the molecular identifications. One mixed culture of *M. avium* and *M. kansasii* yielded a score of 1.548 for *M. avium* with a score of 2.155 for *M. kansasii* upon manual laser operation on the duplicate spot, prompting the suspicion of a mixed culture.

The scores obtained in MALDI-TOF MS experiments reflected the amount of experience of the technician performing the extraction. Two previously trained technicians analyzed the first 35 cultures, which yielded 6 identifications with scores of >2.0 with automated settings (17%) and 16 with scores of >2.0 with manual laser operation (46%), all from primary MGIT cultures. The third technician analyzed the remaining 19 positive cultures, which yielded one identification with a score of >2.0 with automated settings (5%) and none with a score of >2.0 with manual laser operation from primary MGIT cultures. The species distribution was similar in both periods. Scores obtained by the third technician increased over time during the study, reflecting increasing experience.

In this real-life setting, MALDI-TOF MS identified few NTM directly from primary liquid cultures of respiratory samples. Subculture on solid medium and manual laser operation were needed to get good-quality identification results, even if an experimental score threshold of 2.0 for species level identification was applied. The use of two spots per isolate might overestimate the actual performance of the technique. Apart from technician experience, this relatively poor performance directly from newly positive liquid cultures likely reflects three issues. The first is the presence of proteins from the patient or the oropharyngeal flora killed during decontamination, leading to mixed protein patterns. Second, the low number of bacteria present in these early positive cultures and incomplete extraction of proteins may have led to the high number of "no peaks found" results. Finally, we used version 2.0 of the Mycobacteria database, which has since been updated. Because of differences in clinical relevance (2) and treatment regimens between NTM species (1), rapid and accurate identification to the species level is of paramount important for the diagnosis and treatment of NTM pulmonary disease.

Previous studies of the identification of mycobacteria by MALDI-TOF MS used laboratory strain collections, i.e., pure cultures from frozen stocks, or worked only from solid media (4, 7, 8). In that setting, MALDI-TOF MS performs much better, but that is not informative for clinical laboratories, where identification of primary cultures is required to guide diagnosis and treatment. Given the strong effect of technician training, this technique should be performed only by experienced technicians. The need for subculture and manual laser operation significantly increases the amount of hands-on time required to identify an isolate. This voids one of the key benefits of MALDI-TOF MS identification, its low price.

All identifications based on scores of >1.7 were identical to the results of molecular identification. It thus appears reasonable to accept scores of >1.7 as species-level identifications, as for yeasts (9).

In summary, MALDI-TOF MS identification of NTM directly from primary cultures of respiratory samples yields poor results. Only after subculture on solid medium and manual laser operation was a high percentage of the isolates correctly identified. Duplicate testing and experienced technicians are required to maximize the impact of this technique.

FUNDING INFORMATION

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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