Evaluation of Sample Preparation Methods, Instrumentation, and Databases for the Identification of Rapid Growing Mycobacterium using MALDI-TOF MS

Allison R. McMullen, Craig B. Wilen, and Carey-Ann D. Burnham
Washington University in St. Louis, 660 S. Euclid Ave., St. Louis, MO 63110

The identification of *Mycobacterium* spp. is a labor intensive process that historically has required phenotypic testing combined with nucleic acid probe hybridization, HPLC, and DNA sequencing. These approaches can be costly, require specialized equipment, and turnaround time may be prolonged. Matrix-associated laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been shown to be a rapid and accurate method of identification for many types of microorganisms. MALDI-TOF MS for *Mycobacterium* spp. present some unique challenges, including a thick cell wall requiring disruption prior to MALDI analysis, and the need for effective protocols for organism inactivation for biosafety. We evaluated two extraction protocols, two MALDI-TOF MS instruments (Bruker Biotyper and VITEK MS) and the associated databases for each instrument for MALDI-TOF MS identification of rapid growing Mycobacterium (RGM) cultivated on solid media. Sixty-four isolates were evaluated, as listed in Table 1. Sequencing of *rpoB* and/or 16S rRNA gene targets, as appropriate per species, was the gold standard for comparison. A total of three replicates were performed for each experiment using two different operators and the highest overall score was selected as the final identification. The Bruker Biotyper correctly identified 62/64 isolates. Both unidentified isolates (*M. mucogenicum* complex, *M. abscessus* complex) did not provide any results for analyses. Biotyper identifications are accompanied by a confidence "score"; this score is a logarithmic value from 0 to 3 with a score of 3.0 being the best possible score. Of the isolates that were identified, 37/62 (60%) had a maximum score $\geq$ 2.0, 14/62 (23%) had a score between 1.7-2.0 and 11/62 (17%) isolates had a score of $\geq$ 1.7. For the VITEK MS platform, two different databases (Saramis and VITEK MS v3.0) were evaluated. These platforms report identifications as a confidence value, which ranges from 0 to 100%, with 100% being the best possible score. Using the Saramis database, 62/64 isolates were identified correctly. One isolate (*M. abscessus* complex) did not produce any peaks for analyses and one isolate (*M. fortuitum* complex) was incorrectly identified as *Streptococcus anginosus* (confidence value 80.5) in one

of three experimental runs. The other two runs did not produce any peaks and thus could not be analyzed. Of the isolates identified, 58/62 (94%) had a confidence value of ≥ 90.0 and 4/62 (6%) isolates had a confidence value < 90.0. Finally, the VITEK MS v3.0 database correctly identified 60/64 (94%) isolates, with four isolates (two *M. abscessus* complex, one *M. fortuitum* complex and one *M. mageritense*) not producing any data for analyses. Of the isolates identified, 56/60 (93%) had a confidence value ≥ 90.0, while 4/60 (7%) had a confidence value < 90.0.

Both instruments and all three databases tested provided reliable results, with only one incorrect identification in the dataset. The incorrect identification had a low confidence value. The main differences between the instrumentation platforms were with *M. mucogenicum* complex and *M. chelonae* isolates. While both instruments provided accurate identifications for these organisms, the VITEK MS instrument provided consistently high confidence values. Both MALDI-TOF instruments and associated databases had difficulty in differentiating between *M. abscessus* subspecies (*M. abscessus, M. bolletii* and *M. massiliense*), members within the *M. fortuitum* complex (*M. fortuitum, M. porcinum, M. senegalense, M. conceptionense,* and *M. septicum*) and members of the *M. mucogenicum* complex (*M. mucogenicum, M. phocaicum*). For our studies, these were only identified to the complex level.

For RGM, both platforms and all databases provided rapid and accurate identification for the isolates tested. Overall, the VITEK-MS sample preparation required fewer steps and less hands-on time for the RGM isolates tested. There were no major differences between the VITEK MS databases (Saramis, v3.0). Based on our studies, MALDI-TOF MS will be able to expedite the identification of RGM from clinical specimens.

Table 1. Identification of *Mycobacterium* spp. with different MALDI-TOF instrumentation and databases.

Species/Complex (n)	Bruker Biotyper (Score)[1]				VITEK MS Saramis (Confidence Value)[1]			VITEK MS v3.0 (Confidence Value)[1]		
	No score	<1.7	1.7-2.0	≥2.0	No score	<90	≥90	No score	<90	≥90
***M. abscessus* complex[2] (19)**	1	1	3	14	1	2	16	2	1	16
***M. fortuitum* complex[3] (16)**	0	2	0	14	1	1[5]	14	1	2	13
***M. chelonae* (10)**	0	6	4	0	0	0	10	0	0	10
***M. mucogenicum* complex[4] (13)**	1	2	5	5	0	0	13	0	1	12
***M. immunogenum* (4)**	0	0	0	4	0	0	4	0	0	4
***M. mageritense* (1)**	0	0	1	0	0	1	0	1	0	0
***M. neoaurum* (1)**	0	0	1	0	0	0	1	0	0	1
Total (n)	2	11	14	37	2	4	58	4	4	56

[1]*Scores and confidence values represent highest value from three experiments*

[2]*M. abscessus* complex: *M. abscessus* subspecies *abscessus, bolletii, massiliense*

[3]*M. fortuitum* complex: *M. fortuitum, M. porcinum, M. septicum, M. conceptionense, M. senegalense*

[4]*M. mucogenicum* complex: *M. mucogenicum, M. phocaicum*

[5]Incorrect identification (*Streptococcus anginosus*) in one of three experimental runs.