Comparison of the Bruker Biotyper MS and bioMerieux VITEK MS Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometers for Mycobacterial Identification Using Simplified Protein Extraction Protocols

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Background

Matrix assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-ToF MS) has been described as a rapid, cost-effective method of identifying *Mycobacterium sp*. However, the published protocols for mycobacterial protein extraction are complex, using materials not often found in a clinical microbiology laboratory . Additionally, the performance of these protocols on different MALDI-ToF MS platforms has not been established.

We therefore tested two simplified protein extraction protocols, developed at the University of Washington Medical Center (UW) and by bioMerieux, respectively, on the Bruker MicroFlex MS and bioMerieux VITEK MS for identification of *Mycobacterium* species.

Methods

Isolate Selection

•*Mycobacterium* species specifically addressed by the American Thoracic Society official statement on NTM diagnosis and treatment (3) were chosen for analysis.
•*Mycobacterium* isolates were retrieved from frozen stocks. All isolates in the study had been identified by sequencing portions of the hsp65, rpoB or 16S rDNA genes.

Library Creation

•The UW library was created using Bruker protocols for library creation. At least 20 spectra from each organism were combined for each main spectrum (MSP).

Species	N tested	N in library	Species	N tested	N in library
TOTAL	199	125	*M. kansasii*	14	10
M. abscessus ssp. abscessus	17	8	*M. lentiflavum*	10	5
M. abscessus ssp. bolletii	9	4	*M. marinum*	15	9
M. avium	14	9	*M. mucogenicum*	14	10
M. chelonae	14	10	*M. scrofulaceum*	1	1
M. fortuitum complex	12	6	*M. simiae*	9	5
M. gordonae	12	8	*M. szulgai*	9	5
M. haemophilum	4	4	*M. terrae* complex	4	3
M. immunogenum	5	5	*M. tuberculosis*	16	13
M. intracellulare	12	6	*M. xenopi*	8	4

Table 1: Species tested using both protocols and added to the UW library.

Methods (Continued)

Protein Extraction Protocols

UW protocol

1. Transfer 1 µL biomass into a screw top Eppendorf tube with 300 µl of deionized water and approximately 200 µL of 1 mm silica beads.
2. Heat to 95ºC for 30 min.
3. Add 900 µL of 100% ethanol and vortex in a horizontal position for 10 min.
4. Transfer the supernatant to a new tube using a pipette.
5. Centrifuge for 2 min at 14,000x g.
6. Aspirate off as much ethanol as possible then let air dry for 10 min.
7. Add 10 µl of 70% formic acid and mix by repeat aspiration/expulsion.
8. Add 10 µl of 100% acetonitrile and vortex for 20 sec.
9. Centrifuge for 1 min at 10,000x g.
10. Use 1 µl of supernatant for MALDI-ToF MS.

Benefits:
- Fewer steps performed in the biosafety cabinet
- Recovers more specimen

bioMerieux protocol

1. Transfer 1 µL biomass into a screw top Eppendorf tube with 500 µl of 70% ethanol and approximately 200 µL of 0.5 mm silica beads.
2. Vortex in a horizontal position for 15 min.
3. Let sit at room temperature for 10 minutes
4. Transfer the supernatant to a new tube using a pipette.
5. Centrifuge for 2 min at 10,000x g.
6. Aspirate off as much ethanol as possible.
7. Add 10 µl of 70% formic acid and mix by repeat aspiration/expulsion and let sit for 2-5 min.
8. Add 10 µl of 100% acetonitrile and vortex for 20 sec.
9. Centrifuge for 2 min at 10,000x g.
10. Use 1 µl of supernatant for MALDI-ToF MS.

Benefits:
- Faster protocol
- No heating step

Mass Spectrometry

- All extracts tested on Bruker MicroFlex and bioMerieux VITEK MS.
- Spectra were compared against the Bruker Biotyper Mycobacteria 1.0 library (173 MSPs), the bioMerieux SARAMIS library and the combined UW and Biotpier libraries (298 MSPs).
- Species level identification for Bruker and UW library was defined as isolates with scores >2.0 using Bruker Biotyper software 3.0
- Species level identification for SARAMIS library was defined as isolates with scores >90% against species specific super-spectrum, and/or spectra identified using reference spectra

Results

Both protocols produce adequate spectra on the MicroFlex and VITEK mass spectrometers.

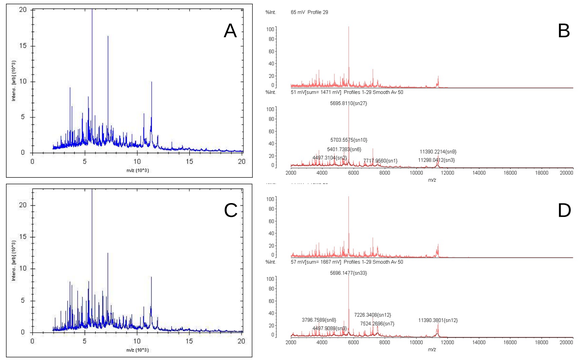


Figure 1: Spectra of *M. kansasii* generated by the Bruker MicroFlex (A,C) and bioMerieux VITEK (B,D) mass spectrometers using the UW protocol (A,B) or the bioMerieux protocol (C,D).

The SARAMIS library outperforms the Biotyper library using either protocol

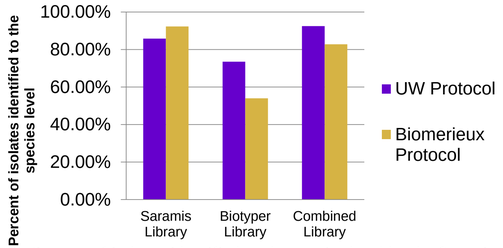


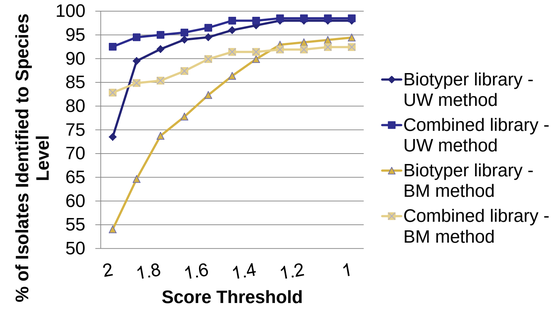
Figure 2: Percent of isolates identified to the species level by the Saramis, Biotyper or UW libraries using the UW or bioMerieux protocols. Spectra generated on the VITEK MS were compared to the SARAMIS library. Spectra generated on the MicroFlex MS were compared to the Biotyper and the UW libraries.

UW protocol
- •UW library 92.5% ID
- •SARAMIS library 85.8% ID
- •Biotyper library 73.5% ID

bioMerieux protocol
- •SARAMIS library 93% ID
- •UW library 82% ID
- •Biotyper library 54% ID

Results (Continued)

Adjusting the score threshold improves species level identification



Discussion

- The UW and bioMerieux protocols were significantly simpler than previously published protocols.
- Both protocols successfully identified isolates representing 18 clinically relevant *Mycobacterium* species.
- The Saramis library outperformed the Biotyper library using either protocol when isolates are identified according to manufacturer specifications.
- The Biotyper library could be used to successfully identify 89.5% of isolates using the UW protocol if a score threshold of 1.9 was accepted. This did not result in false positive identifications.
- Internal library creation allowed for successful mycobacterial identification on the MicroFlex MS platform using either protocol.

References

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2. Saleeb PG, et al. (2011). Identification of Mycobacteria in Solid-Culture Media by Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry. *Journal of Clinical Microbiology* , 49(5): 1790-1794.
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Disclosure