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A VITEK® MS method for direct and rapid identification of *Mycobacterium* species from three automated liquid media systems

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ABSTRACT

For rapid diagnosis of mycobacterial infections, a low-cost identification method performed directly from a positive liquid media culture is needed. The VITEK® MS method requires a 3 mL aliquot of a positive sample that has been incubated for an additional 24-72 hours. The aliquot is transferred into a 5 mL frustoconical tube and centrifuged at 3,000 x g. Residual media is then decanted and the sample blotted dry. The pellet is then re-suspended in 70% ethanol and inactivated through mechanical disruption with sterile glass beads followed by a 10 minute incubation at room temperature[1]. Inactivated mycobacteria are pelleted by centrifugation at 14,000 x g, and residual ethanol is removed. Pellet is re-suspended in 10 µL of 70% formic acid followed by 10 µL of acetonitrile and 1 µL of the final protein extract is deposited onto a MALDI target slide.

Seeded studies were performed with mycobacteria species grown in BacT/ALERT® MP bottles, BACTEC™ MGIT™ 960 tubes, and VersaTREK® Myco bottles and results were analyzed on the VITEK® MS using the V3 database*. A total of 248 out of 251 samples tested were identified as the correct species. No samples were misidentified and 3 samples resulted in no identification.

VITEK® MS method provides direct and rapid identification with reproducible results across multiple automated liquid media systems and successful differentiation of mycobacteria species without any interference from the liquid media.

INTRODUCTION

VITEK® MS is a system for providing bacterial identification using a Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) Mass Spectrometer to analyze the protein profile of a sample and match it to a database of known organism profiles. Identification of mycobacteria isolated from solid media using mass spectrometry has been previously described[2]. However, identifying mycobacteria cultured in liquid media is difficult because there is a lower biomass of microorganisms in the sample and the liquid media may interfere with mass spectrometry.

The described method is designed to remove any interference from liquid media proteins and to have sufficient biomass for consistent and reproducible results in the three most widely used automated liquid media detection systems.

METHODS

- Nine mycobacteria species represented by 33 strains were grown in BacT/ALERT® MP bottles, BACTEC™ MGIT™ 960 tubes, and VersaTREK® Myco bottles using a target inoculum of 5x10^5 CFU per bottle/ tube
- After incubation and positivity in the respective detection systems, samples were further incubated for 24-72 hours to obtain sufficient biomass
- A 3 mL aliquot was transferred into a 5 mL frustoconical tube and centrifuged at 3,000 x g. Residual media was then decanted and the sample was blotted dry
- The pellet was then re-suspended in 70% ethanol and inactivated through mechanical disruption with sterile glass beads followed by a 10 minute incubation at room temperature[1**]
- Inactivated mycobacteria were pelleted by centrifugation at 14,000 x g, and residual ethanol was removed
- Pellet was re-suspended in 10 µL of 70% formic acid followed by 10 µL of acetonitrile
- 1 µL of the final protein extract was deposited onto a MALDI DS target slide

Bibliography/ Acknowledgment:

[1]Totty H et. *al.* 2016. Comparison of mechanical disruption techniques for the rapid inactivation of *Mycobacterium* and *Nocardia* species before identification using MALDI-TOF mass spectrometry. J. Clin. Microbiol.

[2]Girard V. et. *al.* 2016. Identification of *Mycobacterium spp.* and *Nocardia spp.* from solid and liquid cultures by matrix-assisted laser desorption ionization–time of flight mass spectrometry. Diagn. Microbiol. Infect. Dis.

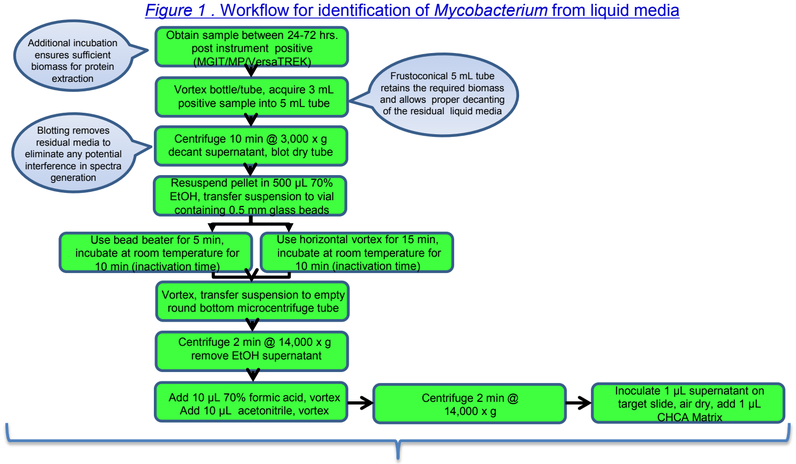
*Consult your local bioMérieux representative for availability of database; dependent upon local regulatory requirements.

**Inactivation studies were performed for mycobacteria using a higher biomass (average population of 1.3 x10^9 CFU) than normally encountered in clinical microbiology.

***Refer to VITEK® MS User Manual for proper routine maintenance and calibration.

METHODS CONTINUED

Figure 1 . Workflow for identification of *Mycobacterium* from liquid media



RESULTS

Nine species of mycobacteria were analyzed on the VITEK® MS*** with an average of three strains per species. Strains were a combination of reference and clinical isolates characterized by molecular methods. Table 1 shows percent identification per species for all media types combined. Each species was correctly identified at ≥ 95% with no mis-identification. Only 3 mycobacteria samples out of 251 (1.2%) resulted in no identification. The data set also included negative samples (data not shown) containing growth and antimicrobial supplements from respective detection systems as well as sputum processing reagents (NALC and NaOH). Spectra generated from negative samples displayed no spectral interference to identification.

Table 1 . Percent identification per species for all media types combined

Species	Number of Samples		Total % Identified
	Species Level ID	No ID	
M. abscessus	15	0	100
M. fortuitum	15	0	100
M. avium	37	2	94.8
M. intracellulare	39	0	100
M. kansasii	33	0	100
M. scrofulaceum	37	1	97.3
M. smegmatis	9	0	100
M. tuberculosis	39	0	100
M. lentiflavum	24	0	100
Total	**248**	**3**	**98.8**

RESULTS CONTINUED

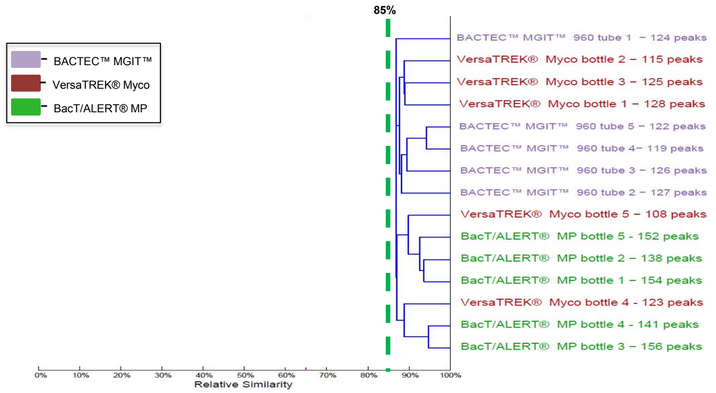
The data set represented in Table 1 was also evaluated for performance per media type with all species combined. Table 2 shows reproducible results across detection platforms with > 97% of isolates being identified to the species level for each media type.

Table 2. Percent identification per media type with all species combined

Media Type	Total # of Samples		Total % Identified
	Species Level ID	No ID	
BacT/ALERT® MP	84	0	100
BACTEC MGIT™ 960	81	2	97.5
VersaTREK® Myco	83	1	98.8
Total	**248**	**3**	**98.8**

Spectra generated by this method were further analyzed to demonstrate results were consistent across all media types. As an example, Figure 2 shows relative similarity between individual spectra of *M. abscessus* isolated from each media type. The method allows for generation of reproducible and consistent spectra regardless of media type with relative similarity of ≥ 85% between spectra.

Figure 2. M. abscessus dendrogram for all media types



CONCLUSIONS

- **Overall, 98.8% of mycobacteria samples were correctly identified on VITEK® MS with performance per species at ≥ 95% and per media type at > 97%**
- **No mis-identifications were observed**
- **Method shows consistent and reproducible results across multiple automated liquid media systems and successfully differentiates mycobacteria species without interference from liquid media components**