27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P079 Mycobacteria and other fastidious microbes

Category: 4b. Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

25 April 2017, 12:30 - 13:30
P1599

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

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Background: For rapid diagnosis of mycobacterial infections, a low-cost identification method performed directly from a positive liquid media culture is needed. Identifying mycobacteria cultured in liquid media is difficult because there is a lower biomass of microorganisms in the sample container and the liquid media may interfere with analytical methods such as mass spectrometry. Here, we describe a cost-effective protocol that allows for direct and rapid identification of mycobacteria from a positive liquid culture using VITEK® MS with total process duration under 45 minutes. The method is designed to remove any interference from liquid media proteins and to have sufficient biomass for consistent and reproducible results for different species of mycobacteria from the three most widely used liquid media detection systems (BacT/ALERT® MP bottles, BACTEC MGIT™ 960 tubes, and VersaTREK® Myco bottles).

Material/methods: Seeded studies were performed with 33 strains representing 9 clinically relevant mycobacteria species grown in BacT/ALERT® MP bottles, BACTEC MGIT™ 960 tubes, and VersaTREK® Myco bottles using a consistent inoculum (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. To pellet mycobacteria from a positive bottle/tube, a 3 mL aliquot was transferred into a frustoconical tube and centrifuged at 3,000 x g. Residual media was then decanted and the sample was blotted dry. The frustoconical tube retains the required biomass and allows proper decanting of the residual liquid media to eliminate any potential interference. 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. Inactivated mycobacteria were pelleted by centrifugation at 14,000 x g, and residual ethanol was removed. Pellet was re-suspended in 10 µL of formic acid followed by 10 µL of acetonitrile. 1 µL of the final protein extract was deposited onto a MALDI target slide.

Results: A total of 251 samples from different mycobacteria species across all media types tested were analyzed on the VITEK MS. 248 samples were identified as the correct species with 99.9% confidence or higher. Three samples resulted in No Identification. Overall, 98.8% of mycobacteria samples were identified as the correct species with 99.9% confidence or higher. No wrong identifications were observed showing the ability of the assay to correctly identify mycobacteria to the species level.

Conclusions: This direct and rapid identification method shows reproducible results across multiple liquid media systems and is successful in differentiating mycobacteria species without any interference from the liquid media.