AFA-based MALDI Biotyper™ Sample Prep for MGIT™ Positive Mycobacteria Cultures

MYCOBACTERIA GROWTH INDICATOR TUBE (MGIT) POSITIVE CULTURES

offer advantages of speed to positivity compared to cultures with separated colonies on solid agar media plates or slants. Also, MGIT instrumentation such as the BACTEC MGIT 960 TB System provides signal automation to the lab that identifies which individual cultures are turning positive. Mycobacteria specialty laboratories and general Clinical Microbiology laboratories are making good use of this instrumentation and media configuration, especially larger facilities. The development of the MALDI Biotyper for identification of Mycobacteria continues to advance the speed of genus/species ID.

FIRST USE OF MALDI BIOTYPER FOR MGIT POSITIVE HAD LOW SUCCESS RATES

Mycobacteria are resilient to lysis and therefore more challenging for peptide extraction to identify by MALDI Biotyper. Covaris Adaptive Focused Acoustics® (AFA®) was first described for sample prep prior to identification of a research strain of *M. smegmatis* (1) and a variety of clinical US isolates in 2015 (2). These results were from colony isolates. It was reported however that MALDI Biotyper identification of Mycobacteria from first-day MGIT positive cultures was only successful on a limited number of samples (3). Applied to the same laboratory species of spiked culture, it was found that MGIT positive Mycobacteria, although flagged as earlier positives, could not be successfully identified with any degree of efficiency.

IMPROVING SIGNAL TO NOISE

At the ASM 2016 meeting, Johns Hopkins Medical Institutes, Baltimore, MD reported (4) on preliminary results to improve MGIT positive cultures identified with Covaris AFA sample preparation for the MALDI Biotyper. The authors noted that longer incubation of the MGIT tube after initially signalling positive by the instrument improved results. The laboratory tested longer incubation of spiked samples by 24, 48 and 72 hours. It was theorized that slow-grower Mycobacteria would require longer re-incubation, whereas fast-grower Mycobacteria could require less re-incubation. By allowing additional replications of the Mycobacteria in liquid, the contributions of both media components and human proteins would be in a lower ratio to the peptides available for extraction by Covaris AFA.

CONCLUSION

Depending on both the sample type that was inoculated to the MGIT broth and the nature of historical growth rate of the species, identification from MGIT positive cultures can be improved to the 70-95% range. Although this study was performed on fewer than 100 isolates, the guidelines recommended by the authors can support laboratories that own the three instruments, MGIT, Covaris Focused-ultrasonicators, and MALDI Biotyper to improve speed of identification and throughput in the Mycobacteria sections of any laboratory.

BIBLIOGRAPHY

1. ECCMID 2015 poster Onigman et.al. Four Minute Mycobacterium spp. Sample Preparation Process for MALDI-TOF Identification.

2. ASM 2015 poster Adams et.al. Five-Minute Ultrasonic Inactivation and Protein Extraction of Mycobacteria for Identification by MALDI-TOF MS.

3. National TB Conference 2015 seminar Tans-Kersten et.al. MALDI TOF for Identification of Mycobacteria.

4. ASM 2016 poster Fisher et.al. Mycobacterial Inactivation/ Extraction/Identification from Positive MGIT Tubes using AFA-Ultrasonication and MALDI-ToF MS

USA: Covaris, Inc. • Tel: +1 781-932-3959 • Fax: +1 781-932-8705 • Email: customerservice@covarisinc.com • Web: www.covarisinc.com
EUROPE: Covaris Ltd. • Tel: +44 (0)845 872 0100 • Fax: +44 (0)845 384 9160 • Email: eucustomerservice@covarisinc.com • Web: www.covarisinc.com
PART NUMBER: M020047 REV A | EDITION JUNE 2016