Algorithm for Rapid Identification of Mycobacterial Species Using Qualitative Real Time PCR and MALDI-TOF

Introduction
The primary goal for a public health Mycobacteriology laboratory is to detect Mycobacterium tuberculosis complex (MTBC) in acid-fast bacilli (AFB) positive cultures. Many clients perform a Nucleic Acid Amplification Test (NAAT) to rule out MTBC prior to sending cultures to the Michigan Department of Health and Human Services (MDHHS) for susceptibility testing, but some are received without any prior testing or indication of MTBC. Additionally, many clients submit commercial broth cultures to MDHHS, but directly testing broths with Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) yields a relatively poor identification profile. It is therefore necessary to develop an algorithm to rapidly screen for the presence of MTBC. Once MTBC has been ruled out, rapid identification of non-tuberculosis mycobacteria (NTM) can be just as crucial for patient diagnosis and treatment.

The algorithm we devised utilizes Qualitative Real Time Polymerase Chain Reaction (qRT-PCR) on broth cultures as a screening tool for the detection of MTBC and Mycobacterium avium complex (MAC) within 24 hours of receipt. A culture identification can then be determined by MALDI-TOF using MicroEx, a single identification call with a score value of ≥ 1.8 or a score value of ≥ 1.7 with two consecutive calls of the same species.

Validation
The MALDI-TOF validation for MTBC and NTM included 144 isolates grown on three different types of solid media (Lowenstein-Jensen slants, Mycobact¨rion 7H11 selective slants, 7H11 isolation plates) and 32 7H9 broth subcultures (n = 176). The validation looked at score values and the number of times a species identification call was given. MDHHS reports a single identification call with a score value of ≥ 1.8 or a score value of ≥ 1.7 with two consecutive calls of the same species.

Solid media identifications showed a 95% agreement with HPLC, genetic probe and biochemical testing methods with a typical turnaround time (TAT) of 24 hours. Mixed culture broths were also tested by creating different ratios of two organisms in one broth. The study showed that a mix of organisms did not produce a different species identification. Depending on the ratios of the organisms, either both organisms were identified or just the predominant species. The 7H9 broth subculture validation compared the MALDI-TOF 7H9 broth identifications to solid media identifications with 100% agreement and a typical TAT of 48-72 hours.

Conclusion
The MDHHS diagnostic algorithm of screening cultures with qRT-PCR to rule out MTBC and follow up with identification by MALDI-TOF provides a new approach for rapid identification of Mycobacterial isolates to aid in patient treatment.

Acknowledgments
MDHHS receives support through the National TB Elimination Cooperative Agreement and the ELC Cooperative Agreement

References