Detection of Mycobacterial Resistance

Franklin Cockerill
Microbiology and Medicine
Mayo Clinic College of Medicine, USA

Mycobacterium tuberculosis accounts for nearly 2 million deaths throughout the world each year.[1] Multi-drug resistant tuberculosis is increasing problematic, contributing to excessive morbidity and mortality.[2] Rapid and reliable methods for identifying *M. tuberculosis* in human specimens and determining antimycobacterial drug resistance is important so that patients receive appropriate therapy as expeditiously as possible.

Conventional approaches for the detection of *Mycobacterium* species and determination of antimycobacterial drug resistance rely on culture-based methods. Refinements in these methods over the years have resulted in automated testing platforms that require less hands-on time and produce results faster. Several automated commercial broth-based systems have replaced the traditional manual plate/tube agar methods. These systems include the BACTEC 460TB and Mycobacteria Growth Indicator Tube (MIGIT) systems (Becton Dickinson, Sparks, MD), the MB/BacT system (bioMerieux, Durham, NC) and the ESP Culture System II (TREK Diagnostics, Westlake, OH). These testing platforms measure differences in carbon dioxide production or oxygen consumption by actively metabolizing mycobacteria and in addition to detection of mycobacteria in human specimens have the capability for determination of drug susceptibility for mycobacteria isolated from specimens. At the time of this writing, the BACTEC 460TB, MIGIT, and ESP Culture System II are FDA-approved for both detection and antimycobacterial susceptibility testing of mycobacteria in the United States. Clinical validations for these systems for antimycobacterial susceptibility testing have been accomplished by head-to-head comparisons with the traditional gold standard, the agar proportion culture method or the first automated method approved by FDA, the BACTEC 460TB system.

For smaller laboratories with lower volumes of tests, antimycobacterial drug testing methods using the Epsilometer Test (Etest, AB BIODISK, Solna, Sweden) may be attractive. However, this method is manual, experience with it is limited and it is not FDA-approved.

Numerous reports have demonstrated the utility of real-time PCR methods for direct rapid detection of *Mycobacterium* spp. in human specimens. Additionally, the same techniques have been shown to be helpful for rapid identification of *M. tuberculosis* genomic mutations associated with antimycobacterial resistance. Real-time PCR testing instruments and reagents are now available from a number of vendors. The ease of use of this technology combined with outstanding performance characteristics (high sensitivity and specificity, and lessoned amplified product contamination risk due to self-contained systems) has established real-time PCR as a new standard for detection of a number of bacterial and

viral pathogens[3].

Several publications indicate the usefulness of real-time PCR for detection of mycobacteria at the genus level[4]. This approach is rapid, objective and sensitive and has the potential to replace the conventional tedious manual approach of acid-fast staining or auramine-rhodamine fluorocrome staining of specimens as a screen for *Mycobacterium* spp. infection. Other studies have shown the utility of real-time PCR for detection of *M. tuberculosis* complex directly in specimens.[5, 6]

A number of studies have validated the effectiveness of real-time PCR for determination of drug resistance in *M. tuberculosis*. As with detection of *M. tuberculosis*, real-time PCR permits determination of drug resistance in hours versus days or weeks required for traditional culture-based methods. A significant drawback is that current assays reported are not comprehensive; they do not identify all resistance phenotypes. Most assays are limited to determination of rifampin and isoniazid resistance associated with previously described mutations in the *rpo*B and *kat*G genes, respectively.[7, 8] A positive result for these mutations indicates resistance; however, a negative result does not rule out resistance. Therefore, for antimycobacterial susceptibility testing, these real-time PCR assays can not replace culture-based methods.

As discussed in the preceding C-3 Abstract, a future possibility for a comprehensive real-time PCR approach for mycobacteria susceptibility determination is molecular growth detection. This method combines limited incubation of mycobacterium cells in broth containing an antimicrobial agent with real-time PCR molecular assessment of growth.[9]

References

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