EDUCATIONAL COMMENTARY – DETECTION AND IDENTIFICATION OF MYCOBACTERIA

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

• Discuss conventional and alternative methods to detect and identify mycobacteria.
• Explain the rationale behind current recommendations for staining, isolating, and identifying mycobacteria.

Tests to detect and identify Mycobacterium tuberculosis complex (MTB) and other mycobacteria now include both conventional and alternative methods. The conventional tests detect visible properties of the organism, such as acid-fastness, colony morphology, and biochemical reactions. By contrast, the newer alternative methods directly detect the genetic composition or other components and products of the organism.

Conventional Methods
Conventional methods to detect and identify MTB and other mycobacteria include acid-fast stains, culture, and biochemical tests.

Acid-fast stains
Three types of stains are used to detect acid-fast bacteria: fluorochrome, Ziehl-Neelsen, and Kinyoun. Of these, the fluorochrome stain is preferred, both because it is more sensitive than the Ziehl-Neelsen or Kinyoun stains, and because fluorochrome-stained smears can be screened at lower magnifications (250X to 400X), which makes screening less time-consuming to perform. Disadvantages are that it requires a fluorescent microscope, and rapidly growing mycobacteria may not fluoresce.

The Ziehl-Neelsen and Kinyoun stains are the classic carbolfuchsin stains. The Ziehl-Neelsen stain, also known as the “hot stain,” uses heat to improve penetration of the stain into the bacterial cell wall. The Kinyoun stain, also known as the “cold stain,” is similar to the Ziehl-Neelsen stain, but it does not require heat.

Culture
Media used to culture mycobacteria fall into 3 categories: egg-based solid media, serum- or agar-based solid media, and liquid media. Each of these categories contains both selective and non-selective formulations. For optimal recovery of mycobacteria, laboratories should choose a combination of culture media which:

1. enhances rapid growth of mycobacteria,
2. allows assessment of colony morphology and pigment production,
3. inhibits growth of contaminants, and
4. is cost effective.
The first goal—enhancing the rate of growth of mycobacteria—can be achieved by using either a liquid medium or the microcolony technique. The use of a liquid medium has been shown to reduce the time to isolate mycobacteria to about 10 days, which is significantly less than the 17 days or longer typical of solid media. Middlebrook 7H12 and 7H13 broths, which are used in the BACTEC automated radiometric culture system (Becton Dickinson Diagnostic Instruments Systems, Towson, MD), are the most sensitive and rapid liquid media. Studies have shown that the BACTEC system can detect *M. tuberculosis* in smear-positive samples as early as 1 week after inoculation.

The microcolony technique has been shown to provide a similar reduction in the time needed to isolate mycobacteria. In this method, a thinly poured Middlebrook 7H11 plate is inoculated, sealed, incubated, and examined under 40X magnification at regular intervals for the appearance of microcolonies.

Besides isolating mycobacteria, culture also provides information that helps identify the organism. Characteristics such as growth rate, pigment production, and colonial morphology place the isolate in a preliminary subgroup. Once the organism has been placed in a subgroup, it can then be identified to the species level by either conventional biochemical tests or alternative tests.

**Biochemical tests**

Biochemical tests to identify mycobacteria include niacin accumulation, nitrate reduction, iron uptake, urease, catalase (semi-quantitative, 68°C), Tween 80 hydrolysis, tellurite reduction, arylsulfatase, pyrazinamidase, growth on thiophene-2-carboxylic acid hydrazide (TCH), and tolerance to 5% NaCl. These tests are well standardized, reproducible, and inexpensive; but they have 2 major limitations. First, they are reliable only for species that have been widely studied, so they may not correctly identify members of newly recognized species. Second, some of these tests are time-consuming, and this can delay definitive results for up to 4 weeks. For these reasons, alternative methods that provide faster and more definitive results now supplement the conventional tests.

**Alternative Methods**

Alternative methods used to identify mycobacteria include chromatography and molecular techniques, such as DNA hybridization and direct nucleic acid amplification tests.

**Chromatography**

Chromatography detects mycolic acids, which are long-chain fatty acids found in the cell walls of mycobacteria. A variety of chromatographic methods have been used, including thin-layer chromatography, gas-liquid chromatography, and high-pressure liquid chromatography (HPLC). Of these, HPLC is most preferred because it has been shown to be a rapid and reliable way to identify many species of mycobacteria. However, it requires considerable expertise, and although the reagent cost per test is reasonable, the equipment is expensive.
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Nucleic acid hybridization
Nucleic acid hybridization techniques are currently available to detect *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. kansasi*, and *M. gordonae*. These tests use DNA probes specific to mycobacterial ribosomal RNA, and they can be used with growth both on solid and in liquid media. Two limitations of the test are that it does not differentiate among the species in *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*), and too much biomass in the test suspension can reduce sensitivity.

Nucleic acid amplification
Two commercially available nucleic acid amplification tests detect *M. tuberculosis* directly from respiratory samples. The Amplified *Mycobacterium tuberculosis* Direct Test (MTD; Gen-Probe®, San Diego, CA) uses a nucleic acid probe to detect rRNA from *M. tuberculosis* complex in concentrated sediments of both smear-positive and smear-negative respiratory samples. Another commercially available test—the Amplicor® *M. tuberculosis* PCR (Roche Diagnostic Systems, Branchburg, NJ)—uses the polymerase chain reaction format to detect *M. tuberculosis* complex only in respiratory specimens with positive acid fast bacillus (AFB) smears. Neither of these tests is approved for non-respiratory specimens, nor will they detect mycobacteria other than *M. tuberculosis* complex. Also, nucleic acid amplification tests often remain positive in specimens from treated patients even after cultures become negative. Consequently, although these tests can enhance a laboratory’s ability to detect *M. tuberculosis* complex, they are not meant to replace conventional methods.

Current Recommendations
Neither the conventional methods nor the newer alternative methods alone satisfy all requirements of definitive identification, rapid results, and cost effectiveness. Also, even if *M. tuberculosis* is identified by an alternative method such as nucleic acid amplification, the organism must still be cultured for susceptibility testing. For these reasons, laboratories now use a combination of conventional and alternative methods to achieve the best balance. Although protocols vary, experts generally recommend that laboratories include a fluorochrome stain for smears, a broth-based or microcolony method for culture, and DNA probes or chromatography for identification.

Suggested Readings

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