Overview of Mycobacterial Culture, Identification, and Drug Susceptibility Testing
1. Essentials for the Mycobacteriology Laboratory: Promoting Quality Practices

1.1 Overview: Mycobacterial Culture, Identification, and Drug Susceptibility Testing

Notes:

Welcome to the Association of Public Health Laboratories Essentials for the Mycobacteriology Laboratory:
Promoting Quality Practices. This module will present an overview of mycobacterial culture, identification and drug susceptibility testing practices.
1.2 Mycobacterial Testing Algorithm

Notes:

This slide depicts the overall testing process from specimen receipt to organism identification and drug susceptibility testing. The specimen is received in the mycobacteriology laboratory and processed. The processed specimen is simultaneously set up for acid-fast bacillus smear, nucleic acid amplification test and culture. Growth is identified and if necessary tested for drug susceptibility.
3. Mycobacterial Culture

Notes:

The overview, purpose and methods of mycobacterial culture.
3.2 Mycobacterial Culture

Mycobacterial Culture

- Gold standard for detection of Mycobacterium tuberculosis complex (MTBC)
- Use of culture increases the number of tuberculosis (TB) cases found over smear alone
  - For MTBC, fewer organisms needed for positive culture than for positive AFB smear
- Culture used for species identification, drug susceptibility testing (DST), and genotyping
- Culture also used to monitor patient response to treatment

Notes:

Mycobacterial culture is the gold standard method for detection of Mycobacterium tuberculosis complex. Using culture increases the number of TB cases identified when compared to using AFB smear alone. Fewer viable organisms are needed for a culture positive than for a smear. In addition, the cultured organism is used for species identification, drug susceptibility testing and genotyping, and can also be used to monitor the patient's response to treatment.
3.3 Culture Media

Notes:

The current recommendations offer labs to use one liquid and one solid media per specimen. Solid media can be either egg-based or auger-based. Liquid—or otherwise known as broth media—are used in automated culture systems. The three FDA cleared systems in the US are the Biomerieux BacT/ALERT® 3D, the BD BACTEC MGIT™ and the Thermo Scientific VersaTREK™. We'll talk more about each of these in the culture module.
3.4 Reporting

Notes:

In general a final report is issued at six to eight weeks post inoculation. Positive reports should be issued as soon as growth has been identified and acid-fast bacilli have been observed. The report should be updated when an identification has been made. At a minimum the report should indicate either Mycobacterium tuberculosis complex or non-tuberculosis mycobacteria have been identified.
3.5 Contamination

Most specimens for AFB testing come from non-sterile sites

- Despite decontamination, some contamination of culture media is to be expected
- Common contaminants include molds, yeast, bacteria, and some NTM

Acceptable contamination rate for liquid media is 5-8% and 3-5% for solid media

Notes:

Since most specimens are from non-sterile sites, contamination is common despite decontamination efforts. Common contaminants can be mold, yeast, bacteria or non-tuberculosis mycobacteria. Acceptable contamination rates for liquid media are five to eight percent. Solid is three to five percent. Observed rates different from the expected ranges should be investigated.
3.6 Biosafety Recommendations for Manipulations of Mycobacterial Cultures

Notes:

According to the current edition of the BMBL and the 2012 MMWR report published by the bio safety blue ribbon panel all procedures for the isolation of Mycobacterium tuberculosis complex, must be done in the BSL-3 laboratory. This includes culture manipulation and propagation. BSL3 practices must include containment equipment, minimization of aerosol production and use of respiratory protection.
4. Mycobacterial identification

Notes:

Mycobacterial Identification.
4.2 Identification of Mycobacteria

Notes:

Prompt and accurate identification of both Mycobacterium tuberculosis complex and non-tuberculous mycobacteria are important for patient management and public health response. Identification results can be used for diagnosis of clinically significant disease, decisions on respiratory isolation and initiation and discontinuation of contact investigations.
4.3 National TB Laboratory

Notes:

The 2012 APHL national TB laboratory services survey of all of the labs currently performing TB testing demonstrated 100 percent of them are performing AFB microscopy. 81.7 percent are performing culture. 37.1 percent are performing identification of Mycobacterium tuberculosis complex, and 16.2 percent is performing first line drug susceptibility testing. 93 percent of public health labs and 26 percent of clinical labs are performing Mycobacterium tuberculosis complex identification.
4.4 Clinical Significance of MTBC and NTM

Clinical Significance of MTBC and NTM

- While some NTM can cause disease, not all NTM isolation is clinically significant
- Identification of MTBC is the most important finding in the laboratory and has serious clinical and public health consequences
- Accurate and timely identification of mycobacteria is crucial
  - Use a multi-faceted approach that includes a rapid identification and phenotypic assessment before issuing a final report

Notes:
Unlike with non-tuberculous mycobacteria, the laboratory diagnosis of Mycobacterium tuberculosis complex is the most important finding in a clinical mycobacteriology laboratory. The finding of this species has vital epidemiologic and public health consequences. Given its clinical significance, its detection should be a primary focus of the laboratory. It is not found in the environment therefore isolation almost always signifies disease. Accurate and timely identification is crucial. The multifaceted approach including a rapid identification and phenotypic assessment should be conducted before a final report is issued.
4.5 Identification Methods

- Classical methods: Growth characteristics and conventional biochemical reactions
- Rapid methods:

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>GenProbe® Accuprobe®</td>
<td>Identifies four common mycobacteria; Most common method used; FDA-cleared</td>
<td>No nucleic acid amplification occurs during this assay; sufficient culture growth is necessary for identification</td>
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<tr>
<td>High Performance Liquid Chromatography (HPLC)</td>
<td>Can identify MTBC and NTM from broth culture and directly from clinical specimens</td>
<td>High equipment costs; FDA-cleared system requires mature solid medium growth; Problems with identification of rapidly-growing mycobacteria</td>
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<tr>
<td>Line Probe assays</td>
<td>Increased sensitivity; Some assays detect mutations for MTBC drug resistance</td>
<td>Can be difficult to differentiate bands; Not FDA-cleared</td>
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<tr>
<td>MALDI-TOF</td>
<td>Rapid identification; Used for many bacteria and fungi in the laboratory</td>
<td>Database limitations; Initial cost investment high; Not FDA-cleared</td>
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<tr>
<td>DNA Sequencing</td>
<td>Quicker turnaround time (TAT); Ability to recognize new strains</td>
<td>High cost; Specialized equipment, expertise and training; Not FDA-cleared</td>
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Current recommendations are to use rapid methods

Notes:

Classical identification methods are comprised of growth characteristics and conventional biochemical reactions. Rapid methods include the FDA cleared GenProbe®, Accuprobe® assay, HPLC and non-FDA cleared line probe assays, MALDI-TOF, and DNA sequencing.
4.6 Recommended Turnaround Time (TAT)

- Identification of MTBC ≤ 21 days from specimen receipt
- Molecular methodologies have dramatically decreased the TAT for identification
- Laboratory workflow and testing practices affect TAT
- Referral of testing can lead to increased TAT

Notes:

Healthy People 2010 and 2020 goals outline the goal turnaround time of TB complex identification from receipt of the specimen as being less than or equal to 21 days. While increased use of molecular methods have decreased turnaround time. Referral of isolates or specimens to reference labs can cause this time to increase.
4.7 Referral of Isolates for Identification

**Referral of Isolates for Identification**

- Reference facilities should be used by laboratories that lack appropriate technologies and resources
  - Healthcare providers and TB Control Programs should be consulted to determine the level of TB laboratory services provided in your jurisdiction
  - Any AFB isolate not identified in-house should be sent within one working day to reference laboratory

**Notes:**

Labs that lack appropriate resources and technologies should refer specimens to reference laboratories. Send outs should be done within one working day.
4.8 Transport

Notes:

Isolates of Mycobacterium tuberculosis complex and positive broths must be sent as Category A infectious substances by properly trained individuals. This is in accordance with Department of Transportation and the International Air Transport Association rules. Patient specimens can be shipped as a Category B biological substance.
5. Growth-based DST of MTBC

Notes:

Growth-based drug susceptibility testing of Mycobacterium tuberculosis complex.
5.2 DST of MTBC

Notes:

Drug susceptibility testing is crucial to TB diagnostics and control. It will dictate the course of treatment for each patient and will provide a patient with their best chance at a cure. It will also indicate a drug resistance and potential new emergence of resistance. And will provide information on appropriate treatment for active TB case contacts. It is also used to estimate the prevalence of primary acquired drug resistance in a community.
5.3 Recommended Panel for DST

- Initial MTBC isolates from ALL patients should be tested for susceptibility against four primary drugs - INH, RMP, EMB, and PZA*

- Isolates resistant to RMP or any two primary drugs should be tested against second-line drugs - Minimally, second-line panel should include amikacin, kanamycin, capreomycin and at least one fluoroquinolone

- DST should be repeated after 3 months if patient remains culture positive

*INH = Isoniazid, RMP = Rifampin, EMB = Ethambutol, PZA = Pyrazinamide

Clinical and Laboratory Standards Institute (CLSI) M24-A2
American Thoracic Society, CDC, IDSA, MMWR 2003, TB Treatment Guidelines

Notes:
All initial positive TB complex isolates from each patient should be tested for susceptibility against isoniazid and rifampin, ethambutol and pyrazinamide. Any isolates resistant to rifampin or any two other primary drugs should be tested against second line drugs, which, at a minimum should include amikacin, kanamycin, and capreomycin and at least one fluoroquinolone. Drug susceptibility testing should be repeated after three months if the patient is still culture-positive.
5.4 DST Performed From Culture

Notes:

An indirect drug susceptibility test is performed after a positive growth is identified as Mycobacterial tuberculosis complex. Cultures must be pure. Mixed cultures can lead to false resistance. Broth should be subcultured to 7H10 or 7H11 for purity checks. Mixed cultures should be subcultured again in an attempt to isolate the TB complex organism.
5.5 Growth-based Methods for DST

Notes:

There are a few growth based methods for drug susceptibility testing. BD MGIT and VersaTREK are tube-based FDA cleared methods. Indirect agar proportion is a plated media based on non-FDA approved lab-developed tests. And sensititor is a non-FDA approved liquid broth system in a 96-well plate format.
5.6 Considerations for DST Referral

- If possible, laboratories should refer liquid cultures for DST rather than waiting for growth on solid media.
- Submitting and referral laboratories should be familiar with shipping guidelines for infectious substances.
- Consider the panel of drugs that the referral laboratory tests.
- Submitting laboratories should monitor TAT of the referral laboratory.

Notes:

If the lab must refer an isoslate for drug susceptibility testing, it is preferable to refer the liquid culture tube rather than waiting for growth on solid media. Both labs must be knowledgeable and properly trained in guidelines for shipping infectious substances. The submitting lab should consider the drug panel offered and also should monitor turnaround time of the reference lab.
5.7 Thank You for Participating!

Overview: Mycobacterial Culture, Identification, and Drug Susceptibility Testing

Course complete

Notes:

This concludes the overview of Mycobacterial Culture, Identification and Drug Susceptibility Testing which is part of the series from the Association of Public Health Laboratories Essentials for the Mycobacteriology Laboratory: Promoting Quality Practices. Individual modules are available for each topic presented here. You can refer to those modules for more information.