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

- Process Specimen
- AFB Microscopy
- Inoculate Media
- Culture Positive
- Species Identification
- Drug Susceptibility
- Amplification-based Tests
- Molecular DST
Overview, Purpose, and Methods

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
Culture Media

• Two major categories of media
  – Solid: egg-based and agar-based
  – Liquid: also often referred to as broth media
  • Used with automated systems
  • 3 are FDA cleared in US:
    - Biomerieux BacT/ALERT® 3D
    - Becton Dickinson BACTEC MGIT™
    - Thermo Scientific VersaTREK™

• Most labs use liquid and one type of solid
Reporting

- Negative report issued at 6–8 weeks
  - Automated systems incubate liquid media for 6 weeks, solid for 6-8 weeks before negative
- Positive report as soon as media turns positive and AFB are observed
  - Update report when identification made
  - Minimally, report of identification should indicate either MTBC or non-tuberculous mycobacteria (NTM)
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
Biosafety Recommendations for Manipulations of Mycobacterial Cultures

• All procedures for isolation of MTBC including culture propagation and manipulation of the cultures are performed in BSL-3 facilities

• Essential practices for manipulation of MTBC cultures:
  – use of containment equipment (e.g., biosafety cabinet, centrifuge safety cups)
  – Minimization of aerosol production
  – use of respiratory protection
Overview, Purpose, and Methods

MYCOBACTERIAL IDENTIFICATION
Identification of Mycobacteria

- Accurate and prompt identification is important for patient management and public health response

- Identification results are used for
  - Diagnosis of clinically significant disease
  - Respiratory isolation decisions
  - Initiating or discontinuing contact investigations
National TB Laboratory Services Survey

580 (100%) labs perform AFB smear microscopy

474 (81.7%) perform culture

215 (37.1%) perform MTBC identification

94 (16.2%) perform first-line DST

- 93% of Public Health Laboratories
- 26% of Clinical Laboratories

APHL. National TB Laboratory Services Survey Report. 2012
Clinical Significance of MTBC and NTM

• Identification of MTBC is the most important finding in the laboratory and has serious clinical and public health consequences

• While some NTM can cause disease, not all NTM isolation is clinically significant

• 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
Identification Methods

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

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<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>
</tr>
<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>
</tr>
<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>
</tr>
<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>
</tr>
<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>
</tr>
</tbody>
</table>
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
    • Submitting laboratories should routinely monitor TAT of the referral laboratory

Tenover, et. Al, 1993; Healthy People 2010 goals
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
Transport

• Isolates of MTBC (including broths known to be positive for MTBC) are considered Category A (Infectious Substances)
• Patient specimens (e.g., sputum) are considered Category B (Biological Substances)
• Transport of both isolates and patient specimens is regulated by the Department of Transportation (DOT) and the International Air Transport Association (IATA) rules
• Persons involved in shipping must be trained and certified since the process is complex and all regulations* must be followed completely

*For details regarding these regulations, please see the information provided in the Reference section
Overview, Purpose, and Methods

GROWTH-BASED DST OF MTBC
DST of MTBC

• Guides choice of chemotherapy—provides the best chance of cure
• Detects drug resistance or confirms the emergence of drug resistance
• Offers insight into appropriate treatment for contacts of patients with active TB
• Used to estimate the prevalence of primary and acquired drug resistance in a community
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
DST Performed From Culture

- Indirect DST is performed after growth is identified as MTBC
- MTBC cultures must be pure; contaminating bacteria can potentially cause false-resistant results
- Broths should be sub-cultured to 7H10/7H11 and blood agar to assess purity and colony morphology
- If a culture is mixed with NTM or other bacteria, laboratories should attempt to re-isolate the MTBC
### Growth-based Methods for DST

<table>
<thead>
<tr>
<th></th>
<th>MGIT 320 or 960</th>
<th>VersaTREK</th>
<th>Indirect Agar Proportion</th>
<th>Sensititre</th>
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<tr>
<td><strong>Company</strong></td>
<td>Becton Dickinson</td>
<td>Thermoscientific</td>
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<tr>
<td><strong>Media</strong></td>
<td>Liquid broth</td>
<td>Liquid broth</td>
<td>Solid</td>
<td>Liquid broth</td>
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<tr>
<td><strong>Format</strong></td>
<td>Tube</td>
<td>Tube</td>
<td>Petri plate</td>
<td>96-well microtitre plate</td>
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<tr>
<td><strong>FDA approved</strong></td>
<td>Yes (cleared)</td>
<td>Yes (cleared)</td>
<td>No (laboratory developed test)</td>
<td>No (research use only)</td>
</tr>
</tbody>
</table>
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
RESOURCES AND REFERENCES
Packing and Shipping Guidance

- IATA Infectious Substances website: http://www.iata.org/whatwedo/cargo/dgr/Pages/infectious_substances.aspx
References

- Review of False-Positive Cultures for Mycobacterium tuberculosis and Recommendations for Avoiding Unnecessary Treatment William J. Burman and Randall R. Reves
- Clinical and Laboratory Standards Institute [CLSI] M24-A2, M48,
- American Thoracic Society, CDC, IDSA, MMWR 2003, TB Treatment Guidelines
References

• Centers for Disease Control and Prevention. “Guidelines for safe work practices in human and animal medical diagnostic laboratories; Recommendations of a CDC-convened biosafety blue ribbon panel”. MMWR 2012;61 (Suppl): 1-102. http://www.cdc.gov/mmwr/preview/mmwrhtml/su6101a1.htm


• ACILT African Centre for Integrated Laboratory Training http://www.cdc.gov/globalaids/resources/laboratory/Lab-Training-Center.html


• APHL. Assessing Your Laboratory, TB Self-Assessment Tool http://www.aphl.org/aphlprograms/infectious/tuberculosis/Pages/TB-Self-Assessment-Tool.aspx