TB Laboratory Testing & Case Studies

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Objectives

- Review the cascade of laboratory tests a clinician may order to diagnose TB disease
- Integrate molecular assays with culture results
- Demonstrate the proper use of TB diagnostic tests using 3 sample cases of TB disease (easy, medium & difficult)

Disclosures

- None
What do all the words mean?

- Prevent Disease
- Promote Wellness
- Improve Quality of Life

Beaumont
Recommended diagnostic options for pulmonary TB

- *See the bugs* [AFB microscopy]
- *Multiply the bugs* [NAATs]
- *Grow the bugs* [cultures]

*Courtesy of Prof. Madhukar Pai, MD, PhD, Mayo TB Center Webinar March 2016*

Mycobacterial Examination

Mycobacterial examination has 6 stages:

1. Proper specimen collection
2. Examination of acid-fast bacilli (AFB) smears
3. Direct identification (NAAT-nucleic acid amplification test)
4. Specimen culturing and final identification
5. Drug susceptibility testing
6. TB genotyping

TB is difficult to diagnose

*Beaumont*
**High Accuracy for Diagnosis of HIV in Contrast to TB DISEASE**

HIV

TB DISEASE

HIV ANTIBODY
HIV RNA

AFB SMEAR

**Sputum studies Michigan 2016**

<table>
<thead>
<tr>
<th>Test</th>
<th>% POSITIVE</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB sputum smear</td>
<td>41%</td>
<td>Negative smear does not rule out TB</td>
</tr>
<tr>
<td>NAAT on AFB+ sputum smear</td>
<td>91%</td>
<td>May be performed on AFB smear negative sputums</td>
</tr>
<tr>
<td>AFB culture confirms M. tb</td>
<td>68%</td>
<td>Gold standard, not always positive</td>
</tr>
</tbody>
</table>

**Specimen Sources**

- **Sputum** (primary)
- Pulmonary aspiration (secondary)
- Body fluids (CSF, pleural, peritoneal, etc)
- Tissue biopsy
- Blood
- Urine
- Gastric aspirate
- Stool (special request)
- Other
Sputum and AFB smears

“See the bugs”

Specimen Collection

Pulmonary Specimen (sputum)

- Early morning specimens = highest yield of AFB
- Collect at least three consecutive specimens at 8-24 hr intervals (at least 1 early morning specimen)
- Recommended volume for testing is 5-10 ml, less may compromise recovery of AFB
- If patient cannot produce sputum by coughing, consider other methods: sputum induction, bronchoscopy, or gastric aspiration
- All persons suspected of TB disease should have sputum cultured

Specimen Collection

- Collect in sterile, leak proof containers
- Seal with tape or parafilm
- Refrigerate specimen to reduce overgrowth of contaminating bacteria during transit to lab / Do NOT refrigerate blood
- Deliver specimen to TB lab within 24 hrs
- Always include patient name on both test request form and the specimen container
Acid-fast Bacilli (AFB) smear
- Least sensitive of all AFB Tests (20-75% positivity)
- Requires 10,000 AFB/ml to be positive
- Positive slide does not differentiate *Mycobacterium tuberculosis* from Non-tuberculosis mycobacteria (i.e. *M. avium*)
- Reported within 24 hours of receiving the specimen in the laboratory

Fluorescent AFB Smear Using Auramine-O Staining
- Very sensitive, takes minutes to read
- Not all that is fluorescent is AFB (need a careful eye)
- Chemical fluorescence, not an immune stain or Direct Fluorescent Antibody
- Can be confirmed with Ziehl-Neelsen (ZN) smear

Nucleic Acid Amplification Test (NAAT) or PCR

“*Multiply the bugs*”
New CDC Guidelines of Use of NAA
MMWR January 16, 2009

- “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.”
- NAAT should be performed on all new AFB+ sputum specimens

MTD-Hologic and Gene Xpert-Cepheid are the only FDA approved methods

Gene XPERT
This is a cartridge based NAAT that can detect the presence of M. tuberculosis complex DNA and resistance to Rifampin.

NAA tests are available that are not FDA approved, such as real time PCR assays
MDHHS performs a real time lab developed NAA test to detect Mtb and MAC using the ABI 7500 FastDX

ABI 7500 FastDX

GentExpert Assay Procedure for the MTB/RIF Test.
AFB Cultures

“Grow the bugs”

AFB Culture Test

- More sensitive than AFB smear
- 10 AFB/ml can produce a positive result vs AFB smear 10,000 AFB/ml
- Culture may be AFB positive even if smear was negative

Tests Performed on Growth in Mycobacteria Culture

- Accuprobe DNA test (not amplified)
- HPLC (high performance liquid chromatography)
- MALDI-TOF
- Biochemical Identification Confirmation
- Drug Susceptibility
MALDI-TOF / HPLC / Accuprobe

- Matrix-Assisted Laser Desorption Ionization - Time of Flight
- Extraction time ~2 hours
- Run time on the instrument approx. 1 minute
- High Performance Liquid Chromatography
- Extraction time ~2 hours
- Run time per specimen ~75 minutes
- M. tuberculosis complex
- M. avium complex
- M. kansasii
- M. gordonae
- Results in ~2 hours

Susceptibility Testing of M. tuberculosis

When to test
- All new M. tb isolates
- Repeat after 90 days of therapy, if specimens continue to produce M. tb
- Relapse or failed therapy

Additional Molecular Tests for TB
CDC – Molecular Detection of TB Drug Resistance (MDDR)

- Rapid testing for DNA mutations associated with drug resistance
- NAAT (+) sputum specimens or culture isolates (prior approval)
- Must meet the following criteria:
  - Known Rifampin resistance
  - Known MDR
  - High risk of Rifampin resistance or MDR-TB
  - High profile patient (e.g. daycare worker, nurse)
  - Mixed or non-viable culture
  - Drug Adverse reaction (e.g. Rifampin allergy)

CDC MDDR

- **First-line** MDDR to detect MDR-TB
  - *rpoB* (Rifampin)
  - *inhA* and *katG* (Isoniazid)
- **Second-line** MDDR to detect XDR-TB
  - *gyrA* (Fluoroquinolones)
  - *rrs* (Kanamycin, Amikacin, Capreomycin)
  - *eis* (Kanamycin)
  - *tlyA* (Capreomycin)
  - *pncA* (Pyrazinamid)
  - *embB* (Ethambutol)

TB DNA Genotyping Universally offered by CDC

Genotyping provides a fingerprint of each isolate
Michigan performs MIRU-VNTR testing, CDC performs the Spoligo testing:

- Used with traditional investigations, genotyping can:
  - Identify outbreaks not previously recognized
  - Confirm/detect transmission
  - Identify risk factors for recent infection
  - Demonstrate re-infection with different strains
  - Detect possible lab cross-contamination
### Demographics of Selected Genotype Clusters in Southeast Michigan, 2008 – 2012

<table>
<thead>
<tr>
<th>Genotype Cluster</th>
<th>n</th>
<th>Race</th>
<th>Ethnicity</th>
<th>Homeless</th>
<th>Alcohol</th>
<th>Drug</th>
<th>Incarceration</th>
<th>HIV positive</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR00012 (MI_0002)</td>
<td>58</td>
<td>63% African-American</td>
<td>18% White</td>
<td>37%</td>
<td>32%</td>
<td>42%</td>
<td>0%</td>
<td>58%</td>
<td>0%</td>
</tr>
<tr>
<td>PCR00291 (MI_0008)</td>
<td>48</td>
<td>97% African-American</td>
<td>3% Hispanic</td>
<td>44%</td>
<td>35%</td>
<td>29%</td>
<td>0%</td>
<td>19%</td>
<td>0%</td>
</tr>
<tr>
<td>PCR04678 (MI_0047)</td>
<td>23</td>
<td>100% African-American</td>
<td>0% Hispanic</td>
<td>27%</td>
<td>27%</td>
<td>48%</td>
<td>9%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*All clusters were majority 45 – 64 yrs of age, male and US-born.

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**3 Sample Cases**

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### Case #1 EASY

From: Current Approaches to Tuberculosis in the United States

*Figures and Legends:*

- Admission chest radiograph showing bilateral lung infiltrates with prominence in the right upper lobe and lingula of the left lung.
### Case MEDIUM

#### 57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³

<table>
<thead>
<tr>
<th>APRIL 2016 “MEDIUM” CASE</th>
<th>1 HIV+ TB suspected</th>
<th>2 Sputum PPD/IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 AFB smear negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 PPD 0 mm</td>
<td></td>
<td></td>
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<tr>
<td>5 IGRA negative</td>
<td></td>
<td></td>
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<tr>
<td>6 PZA, EMB</td>
<td></td>
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<tr>
<td>7 NAAT positive</td>
<td></td>
<td></td>
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<tr>
<td>8 INH, RIF,</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td></td>
<td></td>
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<tr>
<td>10 11 12 13 14 15 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 18 19 20 21 22 23</td>
<td>Drug susceptibility</td>
<td></td>
</tr>
<tr>
<td>24 25 26 DNA genotype</td>
<td></td>
<td>DNA genotype +</td>
</tr>
</tbody>
</table>

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#2 case MEDIUM

#### 57 yr male (continued)

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³

<table>
<thead>
<tr>
<th>APRIL 2016 “EASY” CASE</th>
<th>1 TB suspected</th>
<th>2 Sputum PPD/IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 AFB smear positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 PPD 15 mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 NAAT positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 INH, RIF, PZA, EMB</td>
<td></td>
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<td>7</td>
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<td>8 9</td>
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<td>10 11 12</td>
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<td>13 14 15 16</td>
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<tr>
<td>17 18 19 20 21 22 23</td>
<td></td>
<td>Drug susceptibility</td>
</tr>
<tr>
<td>24 25 26 DNA genotype</td>
<td></td>
<td>DNA genotype +</td>
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</table>
Case #3
Difficult

- Patient from Africa
- History of 3 prior episodes of pulmonary TB
- Coughing, sick again

#3 case MDR suspect

<table>
<thead>
<tr>
<th>APRIL 2016</th>
<th>“DIFFICULT” CASE</th>
<th>1 MDR-TB suspected</th>
<th>2 Sputum IGRA</th>
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<tbody>
<tr>
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<tr>
<td>3</td>
<td>AFB smear positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>IGRA positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>NAAT positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>INH, RIF, PZA, EMB, ???</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>MDR-TB suspected</td>
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<tr>
<td>8</td>
<td>MDR from CDC positive*</td>
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<td>9</td>
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<tr>
<td>10</td>
<td>11 MDR regimen started</td>
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<td>30</td>
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</table>
**MDHHS Lab Confirmation of 2nd Line Drugs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>INH</td>
<td>R</td>
</tr>
<tr>
<td>Rifampin</td>
<td>R</td>
</tr>
<tr>
<td>PAS</td>
<td>R</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>R</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>S</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>R</td>
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<tr>
<td>Streptomycin</td>
<td>S</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>S</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>S</td>
</tr>
<tr>
<td>PAS</td>
<td>S</td>
</tr>
</tbody>
</table>

**IN CONCLUSION**

- **See the bugs** [AFB microscopy]
- **Multiply the bugs** [NAATs]
- **Grow the bugs** [cultures]
- **Kill the bugs**