Texas DSHS-Austin TB Laboratory Services

Denise Dunbar, Ken Jost, Jan Owen, Claudia Sandoval
Texas Dept. of State Health Services
July 28, 2016
Mycobacteriology Lab Diagnostic Methods for TB

- AFB Smear
- Nucleic acid amplification
- AFB Culture
**DSHS-Austin TB Testing Algorithm**

1. **Process Sputum Specimen**
   - 2 Days

2. **Nucleic Acid Amplification Test - NAAT (GeneXpert)**
   - 2 Days

3. **AFB Smear Microscopy**
   - 24 hours

4. **Inoculate MGIT & 7H11 Culture Media**
   - 2-6 weeks

5. **Species identification - HPLC**
   - 2-3 weeks

6. **Drug Susceptibility Testing MGIT 960 & 7H10 Agar Proportion**

7. **Molecular Detection of Rifampin Resistance (GeneXpert)**
   - 2 Days

8. **Molecular Detection of 1st and 2nd Line Drug Resistance (CDC MDDR)**
   - 2 Days
Specimen Quality

• Accurate laboratory results are directly related to the quality of the specimen

• Sputum
  – Recently discharged material from the bronchial tree, with minimal amounts of upper respiratory tract secretions
    • Well coached patient, collect at least 3ml
    • Label tube, form, and indicate test:
      – initial Dx: NAAT
      – Release from respiratory isolation? Order Smear only
      – Drug resistance suspected?

• Transport to lab cool and quickly
Acid Fast Microscopy (AFB Smear)

- Has many qualities of an ideal diagnostic test
  - Rapid & universally available
  - Detects the most infectious cases
  - Used to support diagnosis and identify need to isolate
  - Helps monitor response to therapy
  - Identifies priority cases for nucleic acid amplification (NAA)

- Problems
  - Not sensitive - Misses ~50% of TB
  - Not specific - Positive smear may be NTM
## AFB Smear

<table>
<thead>
<tr>
<th>CAP</th>
<th>ATS</th>
<th>Interpretation</th>
<th>AFB/ml sputum</th>
<th>Infectiousness of patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>&lt;5,000</td>
<td>probably not infectious</td>
</tr>
<tr>
<td>1 or 2 per smear</td>
<td>1 or 2 per smear</td>
<td>weakly positive</td>
<td>~5,000</td>
<td>probably infectious</td>
</tr>
<tr>
<td>&lt;1 per field</td>
<td>1+</td>
<td>moderately positive</td>
<td>~10,000</td>
<td>probably infectious</td>
</tr>
<tr>
<td>1-10 per field</td>
<td>2+</td>
<td>moderately positive</td>
<td>~100,000</td>
<td>probably infectious</td>
</tr>
<tr>
<td>&gt;10 per field</td>
<td>3+</td>
<td>strongly positive</td>
<td>~1,000,000</td>
<td>probably very infectious</td>
</tr>
<tr>
<td>&gt;10 per field</td>
<td>4+</td>
<td>strongly positive</td>
<td>&gt;1,000,000</td>
<td>probably very infectious</td>
</tr>
</tbody>
</table>
Nucleic Acid Amplification Tests (NAAT)

• Tiny amounts of DNA/RNA are amplified (copied) until there is enough for easy detection

• DNA/RNA is examined
  • Identification
  • Detection of Drug Resistance

• Test turnaround time measured in hours
Cepheid GeneXpert MTB/RIF NAAT Test

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result

Time to result, 1 hour 45 minutes

Boehme et al. NEJM 2010
Nucleic Acid Amplification Tests (NAAT)

- Detects *M. tuberculosis* complex nucleic acids; does not distinguish between live and dead bacilli
  - For initial Dx specimens only
  - Not suitable for follow-up specimen or monitoring; cured patients may be NAAT + for years!

- Sensitivity compared to TB culture
  - >95% for AFB smear-positive
  - Only 55-75% for AFB smear-negative

- Does not replace culture for bacteriological Dx
Who Should be Tested?

CDC recommends NAAT on 1\textsuperscript{st} sputum of every person suspected to have TB for whom the test result would alter case management or TB control activities.
How Do I Get a NAAT from the State Lab?

**Section 4. CLINICAL SPECIMEN**

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is this specimen from an outbreak investigation?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient spends substantial time in a congregate setting (e.g. jail, homeless shelter)?</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

- AFB Smear Only (for release from Isolation)
- AFB Smear and Culture
- AFB Smear, Culture and Direct NAAT (Respiratory Diagnostic Specimens Only)
- AFB Smear and Blood Culture

**FOR RESPIRATORY DIAGNOSTIC SPECIMEN, PROCESSED SEDIMENT:**

- Direct NAAT for M. tuberculosis (NAAT ONLY – NO CULTURE PERFORMED)

**Please provide the AFB smear result for this processed sediment:**

**FOR AFB SMEAR POSITIVE SPECIMEN, PROCESSED SEDIMENT:**

- Direct HPLC for Mycobacterium species, not M. tuberculosis

*By Special Request Only. Telephone 512-776-7342 to request this test.*

**Section 5. REFERRED PURE CULTURE**

- Referred AFB Isolate Identification
- MTB Genotyping Only/for Compliance
- Fungal Isolate Identification
- Actinomycete, Aerobic, Identification

**Section 8. SUSCEPTIBILITY TESTING**

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is MDR M. tuberculosis suspected?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Drug susceptibility tests are performed automatically on patient’s initial M. tuberculosis isolate.*

- MTB Primary Drug Susceptibility Panel
  - Ethambutol
  - Isoniazid
  - Pyrazinamide (PZA)
  - Rifampin

- MTB PZA Susceptibility Test Only

- MTB Agar Susceptibility Panel
  - Capreomycin
  - Ethambutol
  - Ethionamide
  - Isoniazid
  - Kanamycin
  - Ofloxacin
  - Rifabutin
  - Rifampin
  - Streptomycin

DSHS automatically performs NAAT on new patient smear positive respiratory specimens
AFB Culture

- More sensitive than smear
  - 5,000 to 10,000 AFB/ml for smear
  - ~10 viable AFB/ml for culture
- Required for drug susceptibilities & genotype
- Positive for only ~85% of Pulmonary TB
  - Requires a quality specimen
  - May be negative due to contamination
- Culture also used to monitor patient response to treatment
- Lengthy
  - 1-6 weeks by liquid media
  - 2-8 weeks by solid media
AFB Culture Media

• Two major categories of media
  • Liquid: also often referred to as broth media
    • Used with automated systems
    • 3 systems are FDA-cleared in US:
      - Biomerieux BacT/ALERT® 3D
      - Becton Dickinson BACTEC MGIT™
      - Thermo Scientific VersaTREK™
  • Solid: egg-based and agar-based
  • Most labs use one liquid and one solid
## M. tuberculosis complex

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Texas strains 2005-2014*</th>
</tr>
</thead>
<tbody>
<tr>
<td>- M. tuberculosis</td>
<td>10,803 (98.3%)</td>
</tr>
<tr>
<td>- M. bovis</td>
<td>142 (1.3%)</td>
</tr>
<tr>
<td>- M. bovis BCG</td>
<td>34 (0.3%)</td>
</tr>
<tr>
<td>- M. africanum</td>
<td>14 (0.1%)</td>
</tr>
</tbody>
</table>

* Data: Texas DSHS Laboratory Genotype Database
False positive are **not rare**

Median false-positive rate = **3.1%** [range 2.2%-10.5%]

Clerical errors were as common as lab errors

Single specimen positive was **sensitive**, but **nonspecific** indicator of false +

- Low colony count (solid medium)
- Long time to positivity (broth medium)

Contact State TB Control Program and request genotype comparison
Drug Susceptibility Testing (DST) of *M. tuberculosis* Complex

Current Recommendations

- Initial isolate should be tested against first-line drugs (FLD)
  - Isoniazid, Rifampin, Ethambutol, Pyrazinamide
  - Repeat test if patient cult+ after 3 mo. Rx
- For isolates resistant to Rifampin or to any 2 FLDs, test second-line drugs
  - Include Fluoroquinolone, Ethionamide, & Injectable (Amikacin, Capreomycin, Kanamycin)
Molecular Detection of Drug Resistance

• Examining DNA of specific genes for mutations known to be associated with phenotypic resistance

• Rapid - analysis takes less than 1 day

• Can be done on culture isolates or directly on NAAT+ specimens
CDC Molecular Detection of Drug Resistance (MDDR)

• Test Indications
  – Known/suspect DR case or contact to DR case
  – Previous TB Treatment
  – Patient from area with high rate of DR TB
  – Large public health impact
  – Mixed or nonviable culture
  – Other
CDC Molecular Detection of Drug Resistance (MDDR)

- Provides 2-3 day DNA sequence analysis for drug resistance prediction
  - 7 classes of anti-TB drugs sequenced
- MDDR complements conventional DST
  - Used alone, MDDR and conventional DST are imperfect
  - Used together, accuracy of drug resistance or susceptibility detection can be improved.
- Conventional DST results are still needed to confirm susceptibility to individual drugs.
Thank you!

Questions/Comments?