Diagnosis of TB: Laboratory
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Nurse Case Management
San Antonio, TX
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Lisa Armitige, MD, PhD has the following disclosures to make:

• No conflict of interests
• No relevant financial relationships with any commercial companies pertaining to this educational activity
**TB Diagnostic Methods**

- AFB Smear
- Nucleic acid amplification
- AFB Culture
- X-ray
- Clinical
- TST
- IGRA

**DSHS-Austin TB Testing Algorithm**

1. Process Sputum Specimen
   - AFB Smear Microscopy: 24 hours
   - Nucleic Acid Amplification Test - NAAT (GeneXpert)
   - Molecular Detection of Rifampin Resistance (GeneXpert): 2 - 6 weeks
   - Inoculate MGIT & 7H11 Culture Media
   - Species identification -HPLC: 2 - 3 weeks
   - Molecular Detection of 1st and 2nd Line Drug Resistance (CDC MDDR)
   - Drug Susceptibility Testing MGIT 960 & 7H10 Agar

   Proportion
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 in low TB prevalence areas (e.g. Texas)
    - Positive smear may be NTM (16% at DSHS-Austin)

- Highly specific where TB is highly prevalent
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
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

- 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 1st sputum of every person SUSPECTED of having TB for whom the test result would alter case management or TB control activities

  - NAAT should NOT be ordered routinely if:
    - Hospital/commercial lab already has NAAT+
    - Clinical suspicion is extremely high, e.g. pt. symptomatic, smear+, Dx=TB, on Rx, i.e. when NAAT+ or – result would not change actions
    - Clinical suspicion is very low, e.g. other Dx probable, spec is to r/o AFB

- Definition of a “suspected TB case” can vary among providers

- TB programs, clinicians, and laboratorians must collaborate to develop policy and procedure for patients to be tested
How Do I Get a NAAT from the State Lab?

DSHS automatically performs NAAT on new patient smear positive respiratory specimens.

How Do NAAT and Culture Compare?

<table>
<thead>
<tr>
<th></th>
<th>NAAT</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT</td>
<td>1-2 days</td>
<td>2-8 weeks</td>
</tr>
<tr>
<td>Initial diagnosis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Detect Non-viable Mtb</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Suitable to Monitor Treatment</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Detect Drug Resistance</td>
<td>Some</td>
<td>Yes</td>
</tr>
<tr>
<td>Detect Drug Susceptible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Genotype for Epidemiology</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
## GeneXpert MTBC Detection Performance
**DSHS-Austin 11/16/12-1/13/14 (1,178 sputum specimens)**

### AFB Smear Positive

<table>
<thead>
<tr>
<th></th>
<th>Mtb Reference Result</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
<td>Accuracy</td>
<td></td>
</tr>
<tr>
<td>Xpert Positive</td>
<td>212</td>
<td>2</td>
<td>214</td>
<td>99%</td>
<td>Sensitivity 99%</td>
<td></td>
</tr>
<tr>
<td>Xpert Negative</td>
<td>3</td>
<td>185</td>
<td>188</td>
<td>99%</td>
<td>Specificity 99%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>187</td>
<td>402</td>
<td>53%</td>
<td>Prevalence 53%</td>
<td></td>
</tr>
</tbody>
</table>

### AFB Smear Negative

<table>
<thead>
<tr>
<th></th>
<th>Mtb Reference Result</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
<td>Accuracy</td>
<td></td>
</tr>
<tr>
<td>Xpert Positive</td>
<td>41</td>
<td>5</td>
<td>46</td>
<td>79%</td>
<td>Sensitivity 79%</td>
<td></td>
</tr>
<tr>
<td>Xpert Negative</td>
<td>11</td>
<td>719</td>
<td>730</td>
<td>98%</td>
<td>Specificity 98%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>724</td>
<td>776</td>
<td>5%</td>
<td>Prevalence 5%</td>
<td></td>
</tr>
</tbody>
</table>

## GeneXpert Rifampin Resistance Performance
**DSHS-Austin 11/16/12-1/13/14 (248 sputum specimens)**

<table>
<thead>
<tr>
<th>Rifampin Phenotype</th>
<th>Total</th>
<th>98% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert Rifampin-R</td>
<td>10</td>
<td>100% Sensitivity</td>
</tr>
<tr>
<td>Xpert Rifampin-S</td>
<td>238</td>
<td>98% Specificity</td>
</tr>
<tr>
<td>Total</td>
<td>248</td>
<td>2% Prevalence</td>
</tr>
</tbody>
</table>

* All Probe E or D

** All Probe B, 4 silent mutations (Phe514Phe), 1 low level RMP-R mutation (Asp516Tyr)
GeneXpert MTB-Rif in Texas

- Negative Predictive Value for Rifampin resistance approx. 100%
  - Predicts RIPE is adequate until growth-based drug susceptibilities become available
    - Cases resistant to INH & EMB only are rare
  - If drug intolerance, predicts FQ efficacy

- Approx. 50% of rifampin resistant predictions are false
  - CDC rapidly (48 hours) confirms or refutes Xpert rifampin resistance prediction

GeneXpert MTB-Rif in the World

Qin et al. Eur Respir J. 2014 Oct 30

- 15,846 instruments & 7.5 million cartridges procured at concessional prices as of 6/30/2014

- 22 HBCs procured 6.4 million (85%). Of those, 4.2 million (66%) of cartridges were procured by South Africa alone, which along with China, India and Brazil, account for 80% of total HBC procurement.

- The ratio smear/Xpert for initial Dx in South Africa was 1.6, significantly lower than most other HBCs where approximately 40–70 smears were performed for each Xpert.

- Wide-scale implementation of Xpert has only occurred in South Africa, while other HBCs continue to rely heavily on smear microscopy.
AFB Culture

• More sensitive than smear
  • 5,000 to 10,000 AFB/ml for smear
  • ~10 viable AFB/ml for culture
  • NAAT negative, culture positive

• Required for drug susceptibilities & genotype

• Positive for only ~85% of PTB
  • 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
  – Solid: egg-based and agar-based
  – Liquid: also often referred to as broth media
    • Used with automated systems
    • 3 are FDA cleared in US:

  • Most labs use liquid and one type of solid
DSHS-Austin MTBC Culture Positive Time to Detection
All (n=5,805) Mtb positive MGIT cultures from 1/1/2010 thru 12/31/2012

<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>

• Rapid methods:

• Classical methods
  Growth characteristics and conventional biochemical reactions

AFB Identification
**M. tuberculosis complex**

- All positive by NAAT & AccuProbe

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

* Data: Texas DSHS Laboratory Genotype Database

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**Tuberculosis Genotyping**

What have been the most useful aspects of universal DNA fingerprinting of MTBC?

- Detecting previously unrecognized cases of transmission
- Reactivation vs. Reinfection
- Detecting *M. bovis* & *M. bovis* BCG
- Detecting false positive cultures
Mtb False Positive Cultures
Burman & Reves, Clin Infect Dis 2000, 31:1390-1395

• 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 lab and request genotype comparison

M. tuberculosis Complex Drug Susceptibility Testing (DST)

• Susceptibility testing based on ability of isolate to grow in medium containing single critical concentration of drug
  • Critical concentration represents lowest concentration that inhibits 95% of “wild” strains (never exposed to drug)
  • Resistance = growth of ≥1% of inoculum in presence of critical concentration of drug
Drug Susceptibility Testing (DST) of *M. tuberculosis* Complex

**Current Recommendations**

- Initial isolate should be tested against first-line drugs (FLD)
  - Isoniazid, Rifampin, Ethambutol, Pyrazinamide

- For isolates resistant to Rifampin or to any 2 FLDs, test second-line drugs
  - Include Fluoroquinolone, Ethionamide, & Injectable (Amikacin, Capreomycin, Kanamycin)
  - Not cycloserine; un reproducible

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### Growth-based Methods for DST

<table>
<thead>
<tr>
<th>Company</th>
<th>Becton Dickinson</th>
<th>Thermoscientific</th>
<th>N/A</th>
<th>Thermoscientific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>Liquid broth</td>
<td>Liquid broth</td>
<td>Solid</td>
<td>Liquid broth</td>
</tr>
<tr>
<td>Format</td>
<td>Tube</td>
<td>Tube</td>
<td>Petri plate</td>
<td>96-well microtitre plate</td>
</tr>
<tr>
<td>FDA approved</td>
<td>Yes (cleared)</td>
<td>Yes (cleared)</td>
<td>No (laboratory developed test)</td>
<td>No (research use only)</td>
</tr>
<tr>
<td>Time to Results</td>
<td>8-14 days</td>
<td>8-14 days</td>
<td>14-21 days</td>
<td>11-21 days</td>
</tr>
</tbody>
</table>
Real World Turnaround Time for MTBC Drug Susceptibility Testing (DST)

- Specimen receipt to 1st line DST by rapid broth: 4 to 5 weeks
- 2nd line drugs: additional 2 to 4 weeks
- Referral to reference labs adds more time
- Molecular methods to detect resistance can help

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
Sensitivity of Drug Resistance Mutations to Detect Drug Resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>% of Resist.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td>rpoB</td>
<td>96%</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>katG &amp; inhA</td>
<td>88%</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB</td>
<td>74%</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>pncA</td>
<td>83%</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA</td>
<td>62%</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>rrs</td>
<td>95%</td>
</tr>
</tbody>
</table>

CDC Molecular Detection of Drug Resistance (MDDR)

- Implemented Sept 2009 for isolates
- Expanded June 2012 for NAA+ specimens
- 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
**CDC Molecular Detection of Drug Resistance (MDDR)**

- Provides 2-3 day DNA sequence analysis for drug resistance prediction
  - Loci for 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, or at least desirable, to confirm susceptibility to individual drugs.

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**Overall Summary**

- Powerful new rapid testing technologies allow laboratories to play a greater role in TB control

- Molecular tests offer rapid, reasonably accurate & predictive results that help fill in a medical & public health management gap that exists between specimen collection & the availability of conventional culture results

- Highly integrated systems-based approaches are essential to realize potential advantages from testing & information technologies
Thank you!

Acknowledgements

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