Nucleic Acid Amplification Testing for the Diagnosis of TB

David Warshauer, PhD
Deputy Director, Communicable Diseases
Wisconsin State Laboratory of Hygiene
19th/20th Century Traditional Algorithm

1. **Process Specimen**
   - 24 hours

2. **AFB Smear Microscopy**

3. **Inoculate Media**
   - 2 to 6 weeks

4. **Species Identification**
   - 2-3 weeks

5. **Drug Susceptibilities**

Courtesy Tom Shinnick, PhD
21st Century Algorithm

Process Specimen

AFB Smear Microscopy
24 hours

Inoculate Media

Species Identification
2 to 6 weeks

Drug Susceptibilities
2-3 weeks

Genetic Tests

Amplification based Tests

Courtesy Tom Shinnick, PhD
Nucleic Acid Amplification Tests

• FDA-cleared for use with respiratory specimens
  • Amplified *M. tb* Direct Test® (MTD): Gen-Probe, Inc.
  • Amplicor® *M. tuberculosis* (MTB): Roche Diagnostics

• Commercial tests available outside US
  • BD ProbeTec™ MTB Direct Detection
  • COBAS® Amplicor® MTB Test
  • COBAS® TaqMan® MTB Test

• Home-brew tests
• Off-label use of FDA-cleared tests
Amplified *M. tb* Direct Test® (MTD):
Gen-Probe, Inc.

- Smear positive and smear negative specimens
- Transcription mediated amplification
- Ribosomal RNA target
  - Multiple copies
- Detection by hybridization protection assay with acridinium ester-labeled MTB complex-specific DNA probe
- No internal amplification control
- Assay time 2.5-3 hours
Amplicor® *M. tuberculosis* (MTB): Roche Diagnostics

- Smear positive specimens only
- PCR
- Target—584-bp region of the gene encoding for 16S rRNA
- Detection by a colorometric reaction of probe-bound biotin-labeled amplified products
- No internal amplification control
- Assay time 4-6 hours
Nucleic Acid Amplification Tests

- Turnaround time of 24 to 48 hours
- Detect *M. tuberculosis* complex NA
- Do not distinguish live and dead bacilli
- Sensitivity
  - >95% for AFB smear-positive TB patients
  - 55-75% of AFB smear-negative, culture-positive TB patients
- Performance improves with increased clinical suspicion of TB
Primary Classification of Participating Laboratories

- Hospital: 38
- Health Department: 35
- Independent: 10
- Other: 1

Number of Laboratories: N=84

Number of Patient Specimens Tested using NAA for M.tb during the Previous Quarter:

- 1-13: 21
- 14-26: 13
- 27-52: 11
- 53-104: 12
- 105-208: 6
- >209: 17

Frequency

Patient Specimens Tested using NAA

Data courtesy Laurina Williams, PhD
Amplification Procedure Used for Direct Detection of *M. tb*

- **Gen-Probe MTD**: 63 laboratories
- **Roche Amplicor**: 13 laboratories
- **In-house**: 9 laboratories

Total number of laboratories: N=85

Courtesy Laurina Williams, PhD
## NAAT Performance – Respiratory Specimens

**Table 1** Pooled values* (95% confidence intervals) of diagnostic odds ratio (DOR), sensitivity, and specificity of five commercial nucleic acid amplification tests (NAATs)

<table>
<thead>
<tr>
<th>Test</th>
<th>NAA method</th>
<th>AFB+</th>
<th>AFB-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOR</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>Amplicor</td>
<td>PCR</td>
<td>117 [56 to 246]</td>
<td>0.96 [0.94 to 0.97]</td>
</tr>
<tr>
<td>Coba Amplicor</td>
<td>PCR</td>
<td>99 [56 to 173]</td>
<td>0.96 [0.95 to 0.97]</td>
</tr>
<tr>
<td>BDIP</td>
<td>SDA</td>
<td>181 [39 to 834]</td>
<td>0.98 [0.96 to 0.99]</td>
</tr>
<tr>
<td>E-MTD</td>
<td>TMA</td>
<td>314 [99 to 995]</td>
<td>0.97 [0.95 to 0.98]</td>
</tr>
<tr>
<td>LCx</td>
<td>LCR</td>
<td>42 [12 to 142]</td>
<td>0.96 [0.94 to 0.98]</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; SDA, strand displacement amplification; TMA, transcription mediated amplification; LCR, ligase chain reaction; DOR, diagnostic odds ratio.

*Random effect model.
<table>
<thead>
<tr>
<th>Year</th>
<th>Culture Confirmed PTB</th>
<th>Smear Positive with MTD</th>
<th>Positive MTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>39</td>
<td>17</td>
<td>16 (94%)</td>
</tr>
<tr>
<td>2007</td>
<td>37</td>
<td>19</td>
<td>19 (100%)</td>
</tr>
</tbody>
</table>
Diagnostic Accuracy of Commercial Tests for TB Meningitis

(A) Commercial tests

Sensitivity

Specificity

56%

98%

## $$$$$$$ Cost $$$$$$$

<table>
<thead>
<tr>
<th></th>
<th>Amplicor®</th>
<th>Gen-Probe®</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagents</strong></td>
<td>3 controls 3 patients $120</td>
<td>2 controls 3 patients $150</td>
</tr>
<tr>
<td><strong>Labor</strong></td>
<td>2.5 hr $50</td>
<td>2 hr $40</td>
</tr>
<tr>
<td><strong>Direct Costs per Patient Result</strong></td>
<td>$57</td>
<td>$63</td>
</tr>
</tbody>
</table>

*Wage of $20/hour
Who should be tested?

- CDC recommends NAAT on first sputum of all patients suspected of TB
- Others suggest not testing patients with low clinical suspicion
- Others suggest not using NAAT when very high clinical suspicion
- Several investigators suggest NAAT be used to confirm TB in patients with intermediate-to-high likelihood of disease. Especially valuable in smear negative patients
Who should be tested (cont)

• Especially valuable in smear negative patients
  – Positive NAAT influences the clinician to start treatment
  – Avoidance of other invasive procedures
  – Avoidance of potentially toxic therapy for other diagnosis
  – Reduction of transmission
Current CDC Recommendations for NAAT

• Collect sputum specimens on 3 different days for AFB smear and mycobacterial culture
• Perform NAAT on the first sputum specimen collected, the first smear-positive sputum specimen, and additional sputum specimens as indicated
  • Number of specimens to tests?
    • Clinical situation
    • Prevalence of TB
    • Prevalence of NTM
    • Laboratory proficiency

MMWR, 2000, 49:593
CDC Algorithm—Smear Positive Sputum

• First sputum smear pos, NAAT-pos
  – Patient presumed to have TB
    ▪ No additional NAAT testing needed

• First sputum smear pos, NAAT-neg
  – Test for inhibitors
    If no inhibitors, additional specimens should be tested (not to exceed 3)
    Presumed NTM if a 2\textsuperscript{nd} smear pos, NAAT-neg
    If inhibitors detected, NAAT no diagnostic help
    Additional specimens can be tested
CDC Algorithm—Smear Negative Sputum

• First sputum smear neg, NAAT-pos
  – Test additional specimens
    ▪ Patient presumed to have TB if a subsequent specimen is NAAT-pos

• First sputum smear neg, NAAT-neg
  – Test additional specimen
    ▪ If NAAT-neg, patient presumed not infectious if smear and NAAT-neg
If repeat NAAT fails to verify initial result
– Rely on clinical judgement for anti-TB therapy, further diagnostic work-up, and isolation

NAAT does not replace AFB smear, culture, or clinical judgement
Challenges to Implementing NAAT

• Reluctance to change
• NAAT adds significant cost to the laboratory
• In current algorithm, NAAT is an add-on test
• The overall costs and benefits of NAAT may vary by program
• Optimal, cost-effective testing regimens have not yet been developed
Are New Algorithms Needed?

• NAAT can provide confirmation within 48 hrs
  • meets a Healthy People 2010 TB goal
• NAAT is becoming standard of care for TB
  • clinicians accept NAAT results
• A positive NAAT result is accepted as laboratory confirmation of a TB case for RVCT
• NAAT can impact treatment and control activities
TB is considered a clinically based diagnosis, but some clinicians delay treatment decisions until laboratory results are available.

Test results must be available as soon as possible to reduce delay in initiation of therapy.

Reduction in turnaround time for laboratory diagnosis of pulmonary TB by routine use of NAAT

Processing: 5 days; NAAT 4 days; broth medium monitored 7 days

NAAT (first specimen) - AFB smear and culture (3 specimens) - 797 pt [81 TB]

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>Mean TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB Smear</td>
<td>70</td>
<td>98</td>
<td>79</td>
<td>96.7</td>
<td>1</td>
</tr>
<tr>
<td>NAAT</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>98.9</td>
<td>2</td>
</tr>
<tr>
<td>Culture x 3</td>
<td>96</td>
<td>100</td>
<td>100</td>
<td>99.6</td>
<td>18</td>
</tr>
</tbody>
</table>

Obtain and Process 3 Clinical Specimens

1 to 2 days

Inoculate Liquid Media

10 to 14 days

Detect Growth

1 to 2 days

Identification – Probes/HPLC

5 to 10 days

Susceptibilities – Liquid Media

NAAT

Microscopy

Current Algorithm

Courtesy Tom Shinnick, PhD
New Algorithm 1

Obtain and Process 3 Clinical Specimens

1 to 2 days

NAAT

Inoculate Liquid Media

10 to 14 days

Detect Growth

1 to 2 days

Identification – Probes/HPLC

5 to 10 days

Susceptibilities – Liquid Media

Microscopy

Courtesy Tom Shinnick, PhD
Obtain 3 Clinical Specimens

Process 1 Specimen
- 1 to 2 days
- NAAT

Process 2 Specimens
- Microscopy
- Inoculate Liquid Media
- 10 to 14 days
- Detect Growth
- 1 to 2 days
- Identification – Probes/HPLC
- 5 to 10 days
- Susceptibilities – Liquid Media

New Algorithm 2

Courtesy Tom Shinnick, PhD
New Algorithm 3

Obtain and Process 2 Clinical Specimens

1 to 2 days

Inoculate Liquid Media

Identification – Probes/HPLC

Detect Growth

1 to 2 days

Susceptibilities – Liquid Media

5 to 10 days

Identification – Probes/HPLC

10 to 14 days

Microscopy

NAAT

1 to 2 days

Courtesy Tom Shinnick, PhD
Decision Algorithm Based on CSTB

- Interpretation of test results depends on the degree of clinical suspicion of TB
  - low CSTB $\leq 25$
  - intermediate CSTB 26–75
  - high CSTB $>75$

- Patients with intermediate or high CST
  - One positive result needed for decisions in

- Patients with a low CSTB
  - Two positive results needed for decisions in
Algorithm for Use of NAAT for Diagnosis of TB

Inoculate media, AFB smears

Collect and process 3 sputum specimens

Perform NAAT on first specimen

Positive

Low CSTB

AFB Smear Positive

Presume Patient has TB

Intermediate or high CSTB

Presume Patient has TB

Negative

Use clinical judgment and other laboratory tests to confirm or exclude TB

AFB smear Negative

Perform NAAT on a second specimen
Algorithm for Use of NAA Tests for Diagnosis of TB

1. **Low CSTB** → **AFB smear Negative** → **Perform NAAT on a second specimen**

   - **Positive**: Presume Patient has TB
   - **Negative**:
     - **Positive**: Use clinical judgment and other laboratory tests to confirm or exclude TB
     - **Negative**: Consider NAAT on 3rd specimen

*Courtesy Tom Shinnick, PhD*
Summary

• Advantages of NAAT
  – More rapid diagnosis
  – Initiation of earlier treatment
  – Cost savings for patient isolation
  – Faster reporting to TB Programs
  – Fewer transmissions
Research Needs for Future Advancements

• Studies to develop, evaluate, and select the most effective and efficient NAAT algorithms
• Develop and evaluate tests for non-respiratory specimens
• Develop tests with improved performance and ease-of-use
• Develop tests that will enhance the diagnosis of TB in children
• Develop multiplex assays that can detect *M. avium* complex, *M. kansasii* and other NTM
Acknowledgements

Tom Shinnick, Ph.D CDC
Julie Tans-Kersten, WSLH

Thank You