TB Detection using Molecular Methods

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Introduction

Microbial Identification and Characterization

**Phenotypic**
- Microscopy
- Biological & biochemical
- Antibiotic & drug susceptibility testing

**Genotypic**
- Probe hybridization
- Nucleic Acid Amplification (NAA)
  - GenProbe MTD
- DNA Fingerprinting
  - Spoligotyping
  - IS6110 RFLP
  - VNTR analysis
Identification for Acid Fast Organisms (MGIT or LJ Culture)

Smear morphology: TB Kinyoun stain

- “Cording” typical of M. tuberculosis
- DNA probe for M.tb complex performed directly from culture tube
- Average time for isolation is 7-21d

- Pleomorphism and branching
- DNA probe for M. avium performed directly from culture tube
- Other Mycobacterium spp. suspected
  - Conventional biochemical
  - Additional probe testing – M. kansasii and M. gordonae

Identification of *Mycobacteria* from Culture using Hybridization Probes (AccuProbe®)

- From Culture ONLY
  - No amplification step
  - Needs lots of target nucleic acid
- Gen-Probe AccuProbes® available for:
  - Mycobacterium tuberculosis complex
    - Mycobacterium tuberculosis, M. bovis (including attenuated BCG), M. africanum, M. microti, M. canetti
  - M. avium complex
  - M. gordonae
  - M. kansasii

Nucleic Acid Hybridization Probes

- Acridinium ester-labeled DNA probes hybridize to *Mycobacterium* specific 16S rRNA target (AccuProbe®)

Nucleic Acid Hybridization Probes

- Acridinium ester on the DNA probe is chemiluminescent
- DNA probe-rRNA hybrids emit light following addition of the detection reagent (hydrogen peroxide/sodium hydroxide)

Nucleic Acid Hybridization Probes

- Chemiluminescence measured in a luminometer and the amount of light emitted proportional to amount of DNA-RNA hybrids formed
- Total time for AccuProbe test is ~2 hours

Sensitivity and Specificity

* M. tuberculosis Complex

<table>
<thead>
<tr>
<th>AccuProbe</th>
<th>CULTURE IDENTIFICATION</th>
<th>Culture</th>
<th>Pos</th>
<th>Neg</th>
<th>Pos</th>
<th>Neg</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Percent Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>422</td>
<td>1</td>
<td>1</td>
<td>541</td>
<td>99.6%</td>
<td>99.1%</td>
<td>99.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 2</td>
<td>185</td>
<td>0</td>
<td>4</td>
<td>213</td>
<td>99.9%</td>
<td>100%</td>
<td>99.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>607</td>
<td>1</td>
<td>5</td>
<td>754</td>
<td>99.2%</td>
<td>99.9%</td>
<td>99.6%</td>
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</tbody>
</table>

When the discordant samples were retested, the correct results were obtained with the exception of one isolate from Site 2 which was nonviable.
Limitations

• False Negatives
  – AccuProbe® test can be negative for *Mtb* complex and still contain TB and eventually test culture positive
  – Why—Number of organisms is below the detectable limit of the test
• Accuprobe testing is FDA-cleared for testing with cultures ONLY

Nucleic Acid Hybridization Probes

<table>
<thead>
<tr>
<th>Mycobacteria</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. avium</em></td>
<td>99.3%</td>
<td>100%</td>
</tr>
<tr>
<td><em>M. intracellulare</em></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><em>M. avium</em> complex</td>
<td>99.9%</td>
<td>100%</td>
</tr>
<tr>
<td><em>M. gordonae</em></td>
<td>98.8%</td>
<td>99.7%</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>92.8%</td>
<td>100%</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> complex</td>
<td>99.2%</td>
<td>99.0%</td>
</tr>
</tbody>
</table>

Courtesy of Nancy Wengenack, Ph.D. (Mayo Clinic)

Nucleic Acid Amplification Tests (NAATs)

• Identify a region of genetic material unique to a particular organism (i.e. *M. tuberculosis*) and amplify this region using DNA replication
• FDA approved the GenProbe Amplified *M. tuberculosis* Direct Test for AFB smear (+) and smear (-) respiratory specimens

Target Genetic Material
Benefits of a Nucleic Acid Amplification Test (NAAT) for TB

• Direct detection with probes is not possible
• Microscopic AFB smears are rapid, but insensitive and non-specific
• Culture is sensitive and specific, but too slow (2-8 weeks)
• Clinical Significance
  • Isolate patients to prevent spread of disease
  • Treatment decisions: Is it Mtb or MOTT?
  • Reduce morbidity and mortality
  • Reduce health care costs for unnecessary isolation/treatment

AMPLIFIED Mycobacterium Tuberculosis (GenProbe MTD) Assay

• Amplified molecular assay detects M. tuberculosis directly from sputum samples in less than 3.5 hours
• Utilizes a Transcription-Mediated Amplification system (TMA) to detect rRNA directly from respiratory specimens
• Limitations:
  • Only detects Mtb complex
  • Negative does not rule out a positive; still need to culture
  • Cross reactions can occur with other rare Mycobacteria

One Mtb organism can contain up to 10,000 copies of rRNA (biological amplification)

AMPLIFIED MTD Test Detects All Members of M. tuberculosis Complex

Mycobacterium africanum
Mycobacterium bovis
Mycobacterium microti
Mycobacterium tuberculosis
Mycobacterium canetti
GenProbe MTD Assay
Transcription Mediated Amplification (TMA)

- Primer hybridizes to target and initiates amplification
- T7 RNA polymerase - transcribes RNA from DNA
- Reverse transcriptase synthesizes DNA from RNA or DNA and has RNAse H activity to degrade RNA after it has been copied into DNA

Contamination Control

Physical separation of pre and post amplification workspaces
Pre amplification - BSL-3 suite
Post amplification - Molecular
- Negative pressure
- Single passage air vented to the outside
Unidirectional workflow
Restricted access
Bio-seal rooms

GenProbe MTD test
FDA-cleared

Approved for:
- Testing smear (+) and (-) specimens (NOTE: Smear (-) specimens NOT tested at DCLS)
- Testing patients who have taken TB medications for LESS than 7 days
- Patients with high clinical suspicion of TB

NOT Approved for:
- Specimens from patients receiving TB medications in the past 12 months
  - NOT a test of cure; MTD can detect nucleic acids from dead and live organisms, so may remain positive long after treatment is completed and the culture is negative
- Testing children or patients unable to produce sputum
Sensitivity and Specificity

- Smear (+) specimens from untreated patients with high suspicion for TB.
  - Sensitivity = 95%
  - Specificity = 98%
- Smear (-) specimens from untreated patients with high suspicion for TB.
  - Sensitivity = 66%
  - Specificity = 98%

Limitations

- Not a perfect test – false positive and false negatives can occur
  - Poor specimen quality
  - Contamination
  - Low numbers of Mycobacterium
  - Inhibited due to a naturally occurring inhibitor in the specimen or processing reagent (e.g., blood)
  - Cross-reactivity (rare!!)
- Does not replace culture results which are the "gold standard".
- Interpret within the context of the patient’s symptoms, chest x-ray, smear and culture

Smear (+) Interpretation

NAAT (+)
- Presume active TB disease
- Start contact investigation
- Start TB medication
- Keep in isolation until cleared
- Confirm by culture

NAAT (-)
- Suspect non-tuberculous mycobacterium (NTM).
- Does not rule out TB
- Consider delaying treatment, contact investigation and removing from isolation UNLESS highly suspected of TB or lives in congregate setting or with high risk individuals request a second NAAT and/or consult TB control.
- Confirm findings with culture
Smear (-)** Interpretation

**NAAT (+)**
- Likely has active TB disease
- Consider submitting another specimen for NAAT
- Presumed TB if two or more specimens are NAAT positive
- Use clinical judgment to determine whether to start treatment, start contact investigation and place on isolation
- Confirm by culture result

**NAAT (-)**
- For smear (-) specimens, sensitivity is low
- Diagnosis of TB cannot be excluded
- MUST rely on clinical judgment
- Consult VDH TB Control to determine if patient can be considered non-infectious if 2 sputum specimens test smear (-) and NAAT results are negative
- Confirm by culture result

**Testing must be pre-approved**

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