Laboratory Services to Support Diagnosis, Treatment, and Control of Tuberculosis

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“Delays in laboratory confirmation of TB and reporting of drug-susceptibility results can lead to delays in initiation of therapy, prolonged infectiousness, inappropriate therapy, and missed opportunities to prevent transmission.”

CDC
National Action Plan to Combat MDRTB, 1992

Today’s Topics

• Overview of TB laboratory services
• Specimen collection and transport
• Detecting and identifying mycobacteria
• Drug susceptibility testing
• New and rapid methods
• Genotyping
TB Laboratory Services

- AFB smear
- Direct identification
- Culture
- Isolate identification
- Drug susceptibility testing
- Genotyping
- Diagnosis of latent TB infection

Laboratory Process

1. Obtain Clinical Specimen
2. Digest, Decontaminate, Concentrate Specimen
3. Inoculate Solid and Liquid Media
4. Detect Growth
5. Identification
   - Probes
   - HPLC
   - Biochemicals
   - Mycobacterium tuberculosis Complex
6. Drug Susceptibility Testing
7. Genotyping
8. AFB Smear
9. Direct Rapid Testing
Laboratory Turnaround Time Goals

- Receive specimen within 24 hr of collection
- Report AFB smear results within 24 hr of specimen receipt
- Detect and identify MTB within 21 days of specimen receipt
- Report drug susceptibility test results within 28-30 days of specimen receipt

CDC Recommendations

For faster Turnaround Time (TAT):
- Specimen transport to lab with 24 hr.
- Fluorescent stains
- Liquid culture
- Rapid ID using high pressure liquid chromatography (HPLC), Probes
- Susceptibility testing using a broth method
- Molecular methods
Laboratory Process

Obtain Clinical Specimen

Digest, Decontaminate, Concentrate Specimen

Inoculate Solid and Liquid Media  AFB Smear  Direct Rapid Testing

Detect Growth

Identification

Probes  HPLC  Drug Susceptibility Testing  Genotyping

Mycobacterium tuberculosis Complex

Biochemicals

The Specimen

Laboratory testing is only as good as the specimen received.

- Is the specimen type appropriate for the site of the infection?
- Is it properly labeled?
- Does the request form have complete, accurate information?
- Is it sent to the lab in timely fashion?
- Is it packaged and transported properly?
<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Comments</th>
<th>Rejection Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchoalveolar lavage or bronchial washings</td>
<td>&gt; 5 ml in sterile leak proof container. Avoid contaminating specimen with tap water. Saprophytic mycobacteria may produce false-positive culture or smear results.</td>
<td>&lt; 5 ml adults &lt; 1 ml pediatric</td>
</tr>
<tr>
<td>Sputum</td>
<td>5-10 ml in sterile, leak proof container. Early-morning specimen from deep, productive cough on at least 3 consecutive days. For follow up of patients on therapy, collect at weekly intervals beginning 3 weeks after initiation of therapy. Expectorated sputum: Instruct patient as to difference between saliva and sputum. Have patient rinse mouth with water before collecting sputum to minimize contamination with food, mouthwash, oral drugs, etc. Induced sputum: Use sterile hypertonic saline. Indicate on request if specimen is induced, as these watery specimens resemble saliva.</td>
<td>1. 24 hour pooled specimens 2. Multiple specimens taken from same day 3. &lt; 5 ml of specimen 4. Expectorated sputum that resembles saliva</td>
</tr>
</tbody>
</table>

**Specimen Transport**

**Specimens**
- Diagnostic sample

**Cultures**
- Infectious material
Laboratory Process

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Inoculate Solid and Liquid Media

Digest, Decontaminate, Concentrate Specimen

Obtain Clinical Specimen

Laboratory Process

Digestion, Decontamination, Concentration

1. Liquefy specimen to release organisms from mucus, cells or tissue
2. Kill or inhibit normal bacterial flora
3. Concentrate

*N-acetyl-L-cysteine

Smear
Culture
NAA
HPLC ID
Molecular ID
Direct AST

1. NALC*
2. NaOH
3. Centrifuge
Acid Fast Smear

• Still an important diagnostic tool
• Rapid and inexpensive
• Procedure:
  – Make smear from concentrated specimen
  – Dry and fix
  – Stain (fluorochrome or carbol fuschin)
  – Wash (decolorize) with acid
  – Counter stain
• “Acid-fast” bacilli (AFB) are not decolorized by the acid wash so retain the first stain,
Acid Fast Smear

• Specificity
  – Not specific for MTb
  – Depends on rate of NTM infection in the population

• Sensitivity
  – 25 – 65% when compared to positive culture but as high as 80% for pulmonary TB
  – Lower for extra-pulmonary TB, childhood TB and non-tuberculous mycobacteria (NTM)
  – 5000 – 10000 bacilli per mL of specimen for “positive” smear
  – Increases when fluorescent stain is used

• # bacilli in pulmonary specimens is directly related to the risk of transmission of TB

Microscope Fields

400X magnification

1000X magnification
Acid Fast Smear

<table>
<thead>
<tr>
<th>RESULT</th>
<th>REPORT</th>
<th>LEVEL OF INFECTIOUSNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB seen</td>
<td>No AFB seen</td>
<td>Potentially</td>
</tr>
<tr>
<td>1-9 AFB/100 fields</td>
<td>Rare, 1+</td>
<td>Lower</td>
</tr>
<tr>
<td>1-9 AFB/10 fields</td>
<td>Few, 2+</td>
<td></td>
</tr>
<tr>
<td>1-9 AFB/field</td>
<td>Moderate, 3+</td>
<td></td>
</tr>
<tr>
<td>&gt;9 AFB/field</td>
<td>Many, 4+</td>
<td>Higher</td>
</tr>
</tbody>
</table>

Mycobacterial Culture

- Sensitive: can detect 1-10 organisms
- Mycobacteria grow SLOWLY
- Enriched culture media promote growth and detection
- Selective media inhibit other bacteria
- Hold cultures 6 – 8 weeks before reporting no growth
- Must culture organism to perform identification, susceptibility testing, and genotyping
Mycobacterial Culture

Solid Media:
- Lowenstein-Jensen
- Middlebrook Agar

Biphasic System:
- Septi-check AFB

Liquid Systems:
- BD BACTEC 460
- BD BACTEC MGIT 960
- BACTEC 9000 MB
- VersaTREK ESP Culture System II
- MB/BacT Alert

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12. Probes
13. HPLC
Detecting Growth

- Solid Media or Biphasic
  - observe visually for growth
- BACTEC, BacT Alert
  - measure released CO₂
- MGIT
  - measure O₂ consumption
- ESP II
  - measure O₂ consumption

Identification of Mycobacteria

- Whenever possible ID to the species level
- Biochemical tests (all species)
- Nucleic acid probes (MTBC, MAC, *M. gordonae*, *M. kansasii*)
- High Pressure Liquid Chromatography (all species)
- DNA sequencing (all species)
Conventional Tests

- Growth rate
- Pigment production
- Biochemical tests
  - Arylsulfatase
  - Catalase
  - Iron uptake
  - Niacin
  - Nitrate
  - Salt tolerance
  - TCH
  - Tellurite
  - Tween 80
  - Urease
  - Etc.

Nucleic Acid Probes

- Available for *M. tuberculosis* Complex, *M. avium-intracellulare* Complex, *M. gordonae*, *M. kansasii*
- Identification of organisms once they have been detected in culture
- Probe “seeks” matching DNA segments in the culture. If it “finds” matching DNA the identification is made.
- Identification in a few hours
HPLC

• Method of identifying types and amounts of mycolic acid
• Identifies Mycobacterium to the species level by comparing mycolic acid patterns to a library of known patterns
• Identification of organism once detected in culture (also direct detection)
• Identification in a few hours

M. tuberculosis

M. kansasii

M. avium cx.
Sequencing

- Examines the 16S rRNA gene
- Compares the sequence to a database of known sequences
- Identifies new species and may be the only way to differentiate some species
- Objective and standardized

![Sequencing Image]

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Drug Susceptibility Testing

- Test the initial isolate from every culture-confirmed TB patient
- First-line testing
  - Streptomycin
  - Isoniazid
  - Rifampin
  - Ethambutol
  - Pyrazinamide
- Now recommend: i I R E and PZA
- Repeat testing if patient fails to respond to therapy or cultures remain positive after 3 months of therapy.

Drug Susceptibility Testing

- Agar proportion
  Reference method
  TAT 21 days
- Broth based systems
  BACTEC, MGIT, TREK
  Growth or no growth
  TAT 6-14 days
- Molecular methods to detect resistance (PCR, sequencing, probes)
Drug Susceptibility Testing

Testing is based on growth or no growth in a medium containing a single critical concentration of each drug.

- Critical concentration is the lowest concentration that inhibits 95% of “wild-type” strains of MTB
- Concentration found to be the best discriminator of known susceptible from known resistant strains for a particular method
- When >1% of the tested bacterial population grows in the presence of the critical concentration of a drug (resistant), that drug is not useful for continued therapy.

Drug Resistance

- MDR-TB is resistant to isoniazid and rifampin
- XDR-TB is resistant to isoniazid and rifampin, plus resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).
- Laboratories will usually confirm resistant results
- Report of an initial resistant result should not be delayed while repeat testing is performed. Lab may report findings as “preliminary”.
- Second-line drug testing should be available (perform or refer) when resistant to 1st line drugs is detected and on request.
MTB Genotyping

- Laboratories in Michigan and California provide testing
- Spoligotyping, MIRU-VNTR, RFLP
- Molecular methods to describe the genetic pattern or “fingerprint” of an MTB isolate
- Matching patterns with epidemiologic link indicates a relationship between the cases

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Healthy People 2010 Goal

Laboratory confirmation of tuberculosis within 48 hours of specimen receipt for 75% of tuberculosis cases that are ultimately culture-confirmed.

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Rapid Direct Methods

• Nucleic Acid Amplification (NAA)
  – Roche Amplicor (PCR amplified test targeting specific segment of 16S rRNA)
  – Gen-Probe MTD (amplification of 16S rRNA followed by probe hybridization)
• Direct HPLC with fluorescence detection
• Direct gene sequencing
• Line-probe assay

Nucleic Acid Amplification (NAA)

• Detects *M. tuberculosis* Complex rRNA directly in clinical sample
• Positive test is very specific
• >95% sensitive if specimen smear-positive
• 70-90% sensitive if smear-negative
• Does not distinguish live and dead organisms
NAA

- Smear pos NAA pos – presumed TB
- Smear pos NAA neg – test for inhibitors
  - If present – NAA is of no help
  - If none and subsequent samples are smear pos and NAA neg - presumed NTM
- Smear neg NAA pos
  - Subsequent sample same – presumed TB
- Smear neg NAA neg
  - Subsequent sample same – presumed not infectious, requires clinical judgement

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July 7, 2000

NAA

- NAA can confirm MTB within 48 hours
- Becoming standard of care
- Significant laboratory cost
- Algorithms for use – clinicians, TB controllers, and labs must work together
Reporting

• Telephone critical results
  – Positive smear
  – Direct Testing
  – Initial culture positive
  – Identification
  – Susceptibility results – especially resistance

• To clinician, ICP, MDH TB control

“The laboratory is an essential part of the diagnosis, treatment, prevention, and control of TB”

Institute of Medicine Report, L. Geitner, ed
Ending Neglect: The Elimination of TB in the US, 2000
Thank you