Laboratory Diagnosis of TB

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March 2017
Objectives

• Describe laboratory methods used for the detection, identification, genotyping, and drug susceptibility testing of *M. tuberculosis*
Outline

• How to collect
• Smear
• Growth-based testing
• Drug Susceptibility testing
When to collect specimens?

• Sputum is the most common specimen
  – Collect 5-10 mls of an early morning specimen, prior to eating

• Usually 3 specimens on 3 different days are recommended for diagnosis
  – At least 8 -24 hours apart
# Why first thing in morning?

<table>
<thead>
<tr>
<th>Study</th>
<th>Specimen Type</th>
<th>Total</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Overnight</td>
<td>160</td>
<td>85,0</td>
</tr>
<tr>
<td></td>
<td>Spot</td>
<td>160</td>
<td>51,8</td>
</tr>
<tr>
<td>2</td>
<td>Overnight</td>
<td>181</td>
<td>31,5</td>
</tr>
<tr>
<td></td>
<td>Spot</td>
<td>179</td>
<td>13,9</td>
</tr>
</tbody>
</table>

1 PANDE et al., Indian J Tuberc 21:1974, 192  
How to collect them?

- https://Sputum Video
What happens after?
Sputum Staining

• 24 hours after receipt of specimen

• Detection limit 5,000 – 10,000 AFB per mL of sputum

• Fluorochrome staining more sensitive than Ziehl-Neelsen
Sputum Staining

- Fluorochrome stains
  - AFB appear yellow against a black background

- Carbol fuchsin-based stains
  - Two types: Ziehl-Neelsen and Kinyoun. (NOTE: ZN is more sensitive)
Direct Detection Using Nucleic Acid Amplification (NAA)

- Rapidly identify a specimen via DNA
- Benefits
  - Earlier lab confirmation
  - Earlier respiratory isolation and treatment initiation
  - Interruption of transmission
- Perform at least 1 NAA per patient
- A single negative NAA does not exclude TB
Culture

- Remains reference standard for confirming diagnosis of TB
- Culture all specimens, even if smear or NAA negative
- Culture monthly until conversion, i.e., 2 consecutive negative cultures
Culture: Solid Media (Agar)

- Middlebrook 7H10 or 7H11 which are agar-based
- Advantage that organisms (colonies) can be seen
- If there is mixed growth or contamination, picking individual colonies can allow you to obtain a pure culture.
7H-10 Media
Culture: Liquid Media

• MGIT 960
  – Results in about 1 week
  – Based on the fluorescence produced from reduced oxygen due to microbial growth
  – Fluorescence then converted to “growth units” (GU).
    • More GU indicates more growth.
    • GU = 400 within, the DST is valid
    • GU<100, the organism is interpreted as being susceptible;
    • GU ≥100, the organism is considered resistant.
MGIT Incubator
Culture: Liquid Media

- VersaTREK: measures change in oxygen due to growth
- Sensititre: 96-well microbroth dilution plate using colonies grown on solid media
- MODS (microscopic observation drug susceptibility): based on visualization of the cording morphology
Culture: Liquid Media

- MGIT 960, VersaTREK, Sensititre
- Results in about 1 week
- Based on the fluorescence produced from reduced oxygen in the MGIT medium due to microbial growth
- The fluorescence generated is then converted to “growth units” (GU). In general, more GU indicates more growth.
- When the growth control generates GU to 400 within 4-14 days, the DST is valid
Drug-Susceptibility Testing

• Conduct drug-susceptibility testing on initial *M. tb* isolate

• Repeat for patients who
  – Do not respond to therapy or
  – Have positive cultures despite 3 months of therapy
  – Retreatment
Methods for DST

• Agar proportion compares growth on agar media with and without one of the four primary drugs

• Broth based (MGIT, Trek)
  – Requires inoculation of the strain in broth with each of the (5) primary drugs, plus control vial
  – Growth of the strain in a vial with a drug indicates resistance to that drug
Drug-Susceptibility Testing
Second-line Drug-Susceptibility Testing

Limit to persons at increased risk for drug resistance:

• Have history of treatment with TB drugs
• Had contact with a person with drug-resistant TB
• Demonstrated resistance to first-line drugs
• Has positive smears or cultures despite 3 months of TB treatment
Genotyping

- Spoligotyping (spacer oligonucleotide typing)
- MIRU/VNTR (mycobacterial interspersed repetitive units/ variable number of tandem repeats)
- RFLP fingerprinting (restriction fragment length polymorphism)
- Whole genome sequencing (WGS)
Spoliogotyping

• Gives a result as a number, so to tell if 2 strains are different, just see if they have different numbers.

• Not too powerful at discriminating different strains.

• Performed at CDC, using DNA sequencer.
Like spoligotyping, the result is a number (24 digits)

Like spoligotyping, MIRU sometimes doesn’t discriminate between unrelated strains

24 locus MIRU protocol is in use, making it more powerfully discriminatory
MIRU and spoligotyping together

- Both PCR-based, so small amount of DNA needed

- If 2 strains of M. tuberculosis are different, unlikely that they will have the same spoligotype AND MIRU type
  - Possible exceptions include Manila strains and Chinese “Beijing” strains
RFLP

• DNA electrophoresis, and requires a lot of DNA
• Complicated procedure that takes ~ a week
• Result is a visual pattern making database input challenging
• Most powerful methods
• Performed only on strains that match by MIRU and spoligo, and only by request
Whole Genome Sequencing

• Other methods interrogate <1% of genome

• WGS looks at entire genome

• Can be used for improved molecular-guided TB surveillance and control to better characterize recent transmission
<table>
<thead>
<tr>
<th>Test</th>
<th>Expected turnaround time (from specimen receipt at laboratory)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB smear</td>
<td>1 day</td>
<td>Fluorochrome staining is more sensitive than carbol-fuchsin acid fast staining (Ziehl-Neelsen or Kinyoun methods).</td>
</tr>
<tr>
<td>Nucleic acid amplification testing (NAAT)</td>
<td>1-2 days</td>
<td>Commercial, FDA-cleared tests and laboratory developed tests available. Excellent sensitivity and specificity for testing smear-positive sediments. Testing smear-negative sediments usually has reduced sensitivity and specificity.</td>
</tr>
<tr>
<td>Molecular detection of drug resistance</td>
<td>1-3 days</td>
<td>Becoming more widely available, particularly for rifampin testing. See Table 5 for more information. New technologies are emerging.</td>
</tr>
<tr>
<td>(may also include identification of M. tb complex)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterial culture and identification</td>
<td>Positive cultures: average of 2-3 weeks incubation. Smear-negative specimens may take &gt;4 weeks to turn positive. 6-8 weeks to report negative.</td>
<td>When a culture takes 5-6 weeks to turn positive, consider investigation for possible cross-contamination.</td>
</tr>
<tr>
<td>Identification of positive cultures</td>
<td>1 day to 1 week for identification of M. tb complex, MAC, M. kansasii, and M. gordonae by DNA probes. Identification of other non-TB mycobacteria may take days or months depending on method used.</td>
<td>Laboratories may batch tests; testing time by DNA probes or MALDI-TOF is less than 2 hours.</td>
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<td>Growth-based DST</td>
<td>Liquid broth systems: 1-2 weeks after setting up DST. (4 weeks or longer from specimen receipt at laboratory.) Solid media (agar proportion method): 3-4 weeks.</td>
<td>DST cannot be performed on mixed or contaminated cultures. Laboratories usually perform DST in batches.</td>
</tr>
<tr>
<td>Genotyping</td>
<td>MIRU: 2 weeks Spoligotype: 1 month</td>
<td>MIRU is performed at the Michigan TB laboratory. Spoligotyping is performed at CDC. Expedited genotyping may be requested for investigation of outbreaks or cross-contamination.</td>
</tr>
<tr>
<td>Interferon gamma release assays (IGRA)</td>
<td>1-2 days (longer if batched)</td>
<td>Usually performed by clinical laboratory (not mycobacteriology laboratory).</td>
</tr>
</tbody>
</table>
Summary

• Don’t forget to get these labs tests for your patients
  – Smear
  – NAAT/Xpert
  – Culture

• Genotyping will be sent by your regional lab