Laboratory Methods: Tuberculosis Diagnosis

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Topics

- Diagnostic testing
  - Smear and Culture
  - Molecular testing
  - Culture-based drug susceptibility testing
- Genotyping

(Reference: Drug-resistant TB: a survival guide for clinicians, 3th ed. Chapter 3 and 1.)
**AFB Workflow**

1 day Specimen processing

AFB Smear

Molecular testing ID Drug resistance

ID 6-8 Weeks Culture

Drug Susceptibility Genotyping

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**Mycobacterium tuberculosis complex**

- Acid-fast bacilli (AFB)
- Slow growing
  - Easily be overgrown by other bacteria.
  - Require special procedures to process
  - Prolonged turnaround time (TAT) for results.
  - Molecular testing significantly shortens TAT.
    - GeneXpert
    - PSQ
**SPUTUM**
*(most common specimen)*

- Best: first morning, deep cough
  - 5 ml (range: 3-10 ml)
  - Sterile, leak-proof container
- Non-sterile source
  - Rinse mouth with water before collecting
  - Do not delay sending to lab. Refrigerate.
- For diagnosis: 3 samples (CDC), 2 (WHO)
  - Collect on different days or at least 8 hr apart.

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**Acid-fast Stains**

- Fluorochrome stains
  - AR (Auramine-rhodamine)
    - Fluorescent microscope.
    - More sensitive than ZN or Kinyoun.
    - Golden orange-yellow
- Carbo fuchsine-based stains
  - ZN (Ziehl-Neelsen) or Kinyoun stain
    - light microscope.
    - AFB--red. Non-AFB--blue
Culture

Media
(Must include solid & liquid media for primary isolation to assure better recovery of various strains)

• Solid media—
  – Egg-based: LJ
  – Agar-based: Middlebrook 7H10, 7H11, etc.

• Liquid media—
  – MGIT (BD)
  – BacT/Alert (bioMerieux)
  – Myco (VersaTrek)

Continuously monitor growth.
Detect growth faster than solid media
MGIT 960

MGIT tubes fluoresce when O₂ is reduced due to microbial growth or other reasons.

LJ slants

7H10 plates
MTBC

Rough colonies on 7H10 medium

Colonial Morphology 
(Microscopic)
Cellular Morphology

MAC  MTB

MTB  Cording Clumps
Culture Identification

- Takes 1-3 weeks to grow
- DNA probes (AccuProbe)
  - MTBC
  - M. kansasii
  - MAC
  - M. gordonae
- MALDI-TOF
- Conventional methods
  - pigmentation, biochemical, growth rate, temperature, etc.
- DNA sequencing

These 4 probes identify ~90% of AFB isolated in clinical labs.

How to speed up diagnosis?

- Molecular testing
  - Identification of MTBC
  - Detection of DRTB
**NAAT**  
*(Nucleic Acid Amplification Test)*

- **FDA-approved:**
  - **MTD**—*Mycobacterium tuberculosis* Direct test
    - Only for identification of MTBC
  - GeneXpert for MTBC & RIF-R detection
- **Not-FDA-approved:**
  - ID of MTBC & drug-R resistance detection
    - Pyrosequencing (PSQ) at MDL
    - Line probes (commercially available)
      - Genotype MTBDR (HAIN)
      - LiPA (INNO)

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**Genes associated with Drug Resistance**

- INH: *katG, inhA, ahpC, fabG1, ndh* (90%), others
- RIF: *rpoB* (>95%)
- Quinolones: *gyrA* (85%), *gyrB*
- Aminoglycosides and CAP: *rrs, eis, tlyA*, etc.
Pyrosequencing (PSQ)

- MDR
- XDR
- Other drug resistance

Pyrogram & PSQ Results

- gyrA mutation in codon 94
- gyrA Wildtype at codon 94

Show sequences. Clear and transparent. No ambiguity.
Mixed Populations

Criteria for Requesting PSQ

- Drug-resistant TB is suspected
  - Immigrants from countries of high DR prevalence
  - Contact of DR
  - Previously treated cases / Not responding to treatment
- Patients have wide exposure or to vulnerable population
- Patients have adverse reactions to INH or RIF
  - Test for 2nd-line drugs
- Laboratory issues
  - Smear-pos but culture-neg for undiagnosed TB
  - Mixed cultures
  - Confirmation of DR from culture-based DST
PSQ for Identification of M. bovis

- Differential identification of:
  - M. bovis
  - MTBC-not-M. bovis
- M. bovis is PZA-R

Impacts on Drug Susceptibility Testing

- Rapid turnaround time hours vs weeks.
- Provide drug results for mixed cultures in most cases.
- Provide ID & DST results for smear-pos but culture-negative cases.
- Rapid confirmation on questionable phenotypic DST results.
  - To rule in resistance, if mutation detected.
Impacts on
TB control & Patient Management

- Early detection of drug resistant TB.
- Early initiation of effective regimen.
- Proper prophylaxis for TB contacts.
- Better TB control.
- Downstream savings.

Clinical Impact on MDR TB

- Based on Molecular beacon assay performed at MDL.
  - Published in JCM (Banerjee, 2010;48:3779)
  - MDR treatment: started 41 days sooner (13 vs 53)
  - Culture conversion: 27 days sooner (63 vs 90)
- PSQ study on clinical impact is in progress.
- Specimens rec’d ~ 10 days after collection.
  - Impact may be greater if specimens are tested sooner.
GeneXpert (Cepheid)

- **Functions:**
  - Identify MTBC & detect RIF-R.
- **Easy to use, fast results**
- **Test raw/concentrated specimens—2 hr.**
- **Performance—**
  - Identification of MTBC—
    - **Sensitivity:** Smear +: 98.2%; Smear -: 72.5%
    - **Specificity:** 99.2%
  - Detection of RIF-R
    - **Sensitivity:** 97.6%
    - **Specificity:** 98.1%

GeneXpert--Limitations

- **Smear-negative specimens**
  - Sensitivity for detecting MTBC (MMWR 2-27-15)
    - Testing single specimen: 55%; 2 specimens: 69%
    - Detection limit: about 100 colonies/ml.
  - False identification of NTM as TB with RIF-R has been encountered.
- **RIF-R: need to be confirmed by sequencing**
  - False RIF-R has been reported
    - Silent mutations are interpreted as RIF-R
    - A common silent mutation detectable by Probe B
## DR Detection

<table>
<thead>
<tr>
<th>Specimen</th>
<th>GeneXpert</th>
<th>PSQ (MDL)</th>
<th>Sanger SQ (CDC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sputum only Raw or sediment Smear-pos or neg</td>
<td>All sources Smear-pos sediments or cultures</td>
<td>All sources Smear-pos sediments or cultures</td>
</tr>
<tr>
<td>TAT</td>
<td>Hours</td>
<td>1 day</td>
<td>1-2 days</td>
</tr>
<tr>
<td>Drug</td>
<td>RIF only</td>
<td>INH, RIF, fQs, AMK, CAP</td>
<td>INH, RIF, EMB, PZA fQs, AMK, CAP, KAN</td>
</tr>
<tr>
<td>Results</td>
<td>Mutations present or not. (Mutation identity not provided)</td>
<td>Wildtype or mutant sequences provided</td>
<td>Wildtype or mutant sequences provided</td>
</tr>
</tbody>
</table>

**Culture-based Drug Susceptibility Testing (DST)**
DST Methods (US)

- Agar proportion ("conventional")—21 days
- Modified proportion methods using liquid media
  - MGIT, 4-14 days
  - VersaTrek
- Sensititre microdilution method, (up to 21 days)
  - Provide MIC results. Cut-point for interpretation not officially established yet.
  - Not available for testing PZA.

Interpretation for Resistance Agar Proportion method

- Resistant:
  - growth in drug quadrant $\geq 1\%$ of growth in the control (no drug).
MGIT 960 DST

• Set up DST on pure cultures.
• Software determines DST completion when control has GU = 400. Drug tube GU <100 = S.
• Visual check MGIT tubes
• Contaminants cause false R
  – Smear: non-AFB or NTM
• Check smear for cording clumps
• Option: Quick confirmation of R by PSQ
  – If mutation not detected, retest on pure culture.

Genotyping

• Epidemiology surveillance
• Investigation of outbreaks
• Investigation of cross-contamination, specimen mislabeling or lab errors.
Genotyping Methods

• PCR-based
  – Spoligotyping (15 digits)
    • Spacer oligonucleotide typing
  – MIRU (12 x 2 loci)
    • Mycobacterial interspersed repetitive unit

• Culture-based
  – RFLP
    • Restriction fragment length polymorphism

• WGS
  – Whole genome sequencing