The Clinician, the Program, and the Mycobacteriology Laboratory

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Effective TB Control depends on an integrated system that includes clinicians, laboratories and TB Controllers

APHL Task Force: The Future of TB Laboratory Services, 2003

Objectives

• To review the role of the Mycobacteriology Laboratory in the Diagnosis and Management of Tuberculosis
  – How the Lab works
  – What the Lab does
  – How to interpret results
• Discuss the potential of new tools

Will not discuss: Molecular Epidemiology Interferon-gamma Release Assays
TB Among the Hobos

- 52 y/o gentleman, traveling (recently to WI) street person
- History of ROH abuse, heavy smoking
- Presents in 4/09 with 2 mos increasing cough, purulent sputum, wt loss
- Questions???
- CXR??

TB – or NOT TB?

- Admitted to MGH
  - Sputum smears AFB-Positive
  - TST: 16mm induration
  - Started 4 drugs: TB Suspect
  - Reported to 1-888-MASS MTB
- Contacts???
- NAAT (MTD™) negative for MTb complex
  - Cultures subsequently grew M. avium
    - Negative for MTb at 8 wk (final)
- Treatment changed to Clarithromycin + Ethambutol
- Patient’s symptoms resolved rapidly

Approach TB Diagnosis

- Clinical presentation
  - History
  - Signs and Symptoms: Site of disease
- Clinical Suspicion is key
  - Personal risk factors for TB
    - Most disease represents reactivation (US)
- Prevalence of TB disease in population
  - Affects predictive values of diagnostic tests
- Patient’s level of immune competence
  - Medical Risk Factors
    - Presentation varies with degree of immunosuppression
TB is a Clinical Diagnosis

*most of the time*

- Most clinicians will initiate multi-drug therapy *if the disease is suspected on clinical grounds*
  - But many cases go undiagnosed until a laboratory reports a positive culture
- How is that diagnosis confirmed?
  - *In the Laboratory*

Role of Mycobacteriology Lab

- **Target:** *Mycobacterium tuberculosis* Complex (Mtbc)
  - Use rapid methods to detect, identify (ID), and perform drug susceptibility testing (DST)
  - *TB vs. not TB*
- **Non-tuberculous mycobacteria (NTM)**
  - Provide accurate / clinically relevant information (accurate ID *IF clinically relevant*; appropriate DST *IF clinically relevant*)
- Issue rapid, clinically useful, and reliable reports
- Evaluate testing and reporting algorithms as necessary
- Develop and maintain 2-way communication - clinicians, care-givers, TB program, referring laboratories, etc.

The TB Laboratory

- **Types**
  - Hospital-Clinical Laboratories
    - Process samples from within an institution and its affiliates
  - Private Laboratories
    - Process samples on contract basis *(e.g. Quest, LabCorp, ARUP)*
  - Network Laboratories
    - Process samples for organization *(e.g. VA)*
  - Public Health Laboratories
    - State/federally supported facilities: *Your State Lab*
  - Reference Laboratories
    - Provide specific services – culture confirmation, molec DST, drug level monitoring, … *(e.g. CDC, National Jewish)*
- Overall, *n >1,932 ( + state labs)*

Accommodating Escalating Complexity

- **Varying Levels of Service Offered**
  - Not all laboratories perform all tests
    - Most perform basic tests: smears, primary cultures
    - Ability to perform appropriate tests
    - Equipment, personnel
    - Secondary and Reference Laboratories
      - Receive/process samples for more complex tests
- **Communication Challenges**
  - Laboratory-to-laboratory
  - Provider-to-laboratory(ies)-to-provider-to- …
- **Laboratory Competence**
  - Determined locally
  - Centers for Medicare & Medicaid Services’ Clinical Laboratory Improvement Amendments (CLIA) program
  - Proficiency testing
Diagnosis of TB: Demonstration of M. tuberculosis

- The Gold Standard
- Secretions or tissue
  - Subjected to laboratory techniques to identify the organism
- Ability to isolate organism varies with
  - Location of Disease
  - Density of organisms at disease site

Standard Mycobacteriology Laboratory Tests

- Smear/stain for acid-fast organisms
  - Sputum, sterile fluids, tissue
- Culture for identification of organism
  - Includes speciation
  - Drug susceptibility studies (DST)
- Nucleic Acid Amplification (NAA)
- Therapeutic Drug Monitoring

Step-by-Step
“Typical” TB smear and culture (1)

- Specimen received in lab
- Specimen accessioned (assigned lab number; entered into lab computer/worklog, etc.)
- Specimen stored appropriately (refrigerated) until processed – usually 1x/workday
- Specimen processed (digested/decontaminated) usually by NALC/NaOH method in batch with other specimens
- Smear prepared
- Culture media inoculated (usually 1 broth and 1 solid) and put into incubator/instrument

Step-by-Step
“Typical” TB smear and culture (2)

- Smear stained and examined and results reported same day as specimen processing
- Nucleic acid amplification (NAA) test set up if appropriate/if lab offers test; some labs also do “molecular DST”
- Culture media examined/monitored as prescribed by method (for 6-8 weeks)
- If growth detected, smear made and stained to confirm presence of AFB (acid fast bacilli)
- If AFB, go onto identification (e.g., HPLC, nucleic acid probe)
- If TB, make appropriate notifications and perform DST as appropriate
- If no growth, keep 6-8 weeks and sign out as “negative for mycobacteria”
Specimen Collection

- **Sputum:** Spontaneous or Induced
  - Initial: 3 good samples, 8-24hr apart (MMWR, 2005)
  - Monthly while on treatment until culture-negative
- **Collect aseptically, avoid contamination**
  - Sterile, leak-proof, disposable, non-breakable, appropriately-labeled lab-approved containers
  - No fixatives or preservatives
- **Avoid contamination with tap water**
- **Collect initial samples prior to therapy if possible**
- **Transport immediately or refrigerate**

**Sputum Smears: Definitions**

- **Direct smear:** stain performed on the submitted sample
- **Concentrated smear:** decontaminated-liquified (NaOH and NALC) and centrifuged (at 3,000xg)
  - Improves yield
  - Procedure kills >30% of mycobacteria
- **Indirect smear:** performed on growth from culture
  - Isolate from primary lab sent to second lab
  - For further identification (confirmation) and drug susceptibility studies
- **Kinyoun or Ziehl-Neelsen (heat) stain:** Light microscopy
  - “Acid-fast”: Organisms retain red color following decolorization with acid-alcohol (the Red Snapper)
- **Fluorochrome stain:** Fluorescence microscopy
  - Recommended initial staining procedure (increased sensitivity, decreased time)

AFB Smear Microscopy

- **Variable sensitivity**
  - 40-70% for pulmonary TB (less in miliary TB, late HIV, children)
  - LOD >10⁴ AFB/ml by Ziehl-Neelsen; >10³/ml fluorochrome
  - Correlates with disease severity and infectiousness
- **Not specific for MTb Complex**
  - Red snappers
- **Inexpensive and quick**
  - Turnaround time (TAT) <24hr
- **Value**
  - Usually provides the 1st evidence of TB
  - Direct smear light microscopy is the primary diagnostic method in developing world
  - Used to guide therapy (AFB in smear are quantified)
  - May guide additional testing (e.g., NAA)

International Guidelines for Examining and Reporting Acid-Fast Smears: Organism Count at Specific Magnifications

<table>
<thead>
<tr>
<th>Report</th>
<th>Number of AFB Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200x, 250x</td>
</tr>
<tr>
<td><strong>No AFB seen</strong></td>
<td>0</td>
</tr>
<tr>
<td>Doubtful: repeat</td>
<td>1-2/30F*</td>
</tr>
<tr>
<td>1+</td>
<td>1-9/10F</td>
</tr>
<tr>
<td>2+</td>
<td>1-9/F</td>
</tr>
<tr>
<td>3+</td>
<td>10-90/F</td>
</tr>
<tr>
<td>4+</td>
<td>&gt;90/F</td>
</tr>
</tbody>
</table>

* number of acid-fast bacilli observed per microscopic field

CDC
AFB Smears: *Rule Out TB?*

- A positive smear does not establish *dx*
- A negative smear does not exclude TB

**Culture Isolation of *M. tuberculosis*:**

The *Gold Standard*

- Requires appropriate laboratory equipment & trained staff: *Competence*
- Allows for identification and speciation, drug susceptibility testing
- Performed on secretions or tissue
- Sensitive
  - Limits of Detection (LOD) 10 to 100 AFB/ml
    - 10,000 AFB/ml for smear (Z-N) - more specimen goes into culture
Culture Methods

• Solid media
  – Agar (Middlebrooks) and egg-based (Lowenstein-Jensen) platforms
  – Require up to 6 - 8 weeks
  – Advantage: Can identify colonies (pigmentation, morphology)

• Broth – some are highly automated
  – BACTEC 460; MGIT; TREK; MB/BacT
  – More rapid recovery than solid media: 7-21 days

• Current recommendations are to use at least one type solid media and broth (mixed culture detection; increased sensitivity)

Colony Morphology

Broth (Liquid Media)
BACTEC 460 Instrument

- Semi-automated; needles
- Laboratory work-horse
- 12B media
- Radiometric
- Detects CO₂ production by mycobacteria
- DST for INH, RMP, EMB, STR, PZA
**Mycobacteria Growth Indicator Tube (MGIT; Broth)**

- Fluorescence quenched by $O_2$ in $O_2$-rich liquid media
- If mycobacteria present, $O_2$ used up, no quench, fluoresces under UV light
- DST for INH, RMP, EMB, STR, PZA

**MTB Culture Isolation**

- Negative cultures do not exclude infectious TB
  - Sampling error, contamination, dead organisms, etc.
- False positive: cross-contamination?
  - Interpretation contextual
  - Depends on clinical suspicion of disease
    - e.g. smear negative, low probability patient
- Cultures guide management
  - Declining # colonies correlate with response to therapy
  - Monitor sputum monthly until culture conversion
  - If culture-pos at 3 mos, look for reason (malabsorption, drug resistance, etc)
- **Rule Out TB?**
  - A positive culture can establish dx
  - A negative culture does not exclude TB

**Identification of Mycobacteria**

- MTb vs NTM: Treatment and Public Health implications
- Preliminary ID based on growth characteristics solid media
  - Colony morphology, pigment, rate of growth (REQUIRES GROWTH)
- Conventional biochemical tests (all mycobacteria)
  - 2-21 d (may not necessarily be accurate for NTMs)
- HPLC of cell wall mycolic acids (”all” mycobacteria)
  - 2 h – usually by reference labs
- Commercially available genetic probes
  - ACCUPROBE, GenProbe, San Diego, CA (www.genprobe.com)
    - probes for Mb Complex, MAC, M. kansasii, M. gordonae
  - 2-4 h – many clinical labs
- “In-house” PCR/genetic sequencing/etc.
  - 1-2 d – reference labs/clinical labs

**Direct Detection of MTB Complex : Nucleic Acid Amplification Testing (NAAT)**

- NAA assays
  - Amplicor®-Roche: DNA
  - MTD®-GenProbe: 1-RNA
- Advantages
  - Excellent sensitivity (10-100 organisms/ml) & specificity for MB
  - TAT generally ≤ 48hr
  - Can affect treatment decisions, including isolation and other public health interventions, invasive procedures
- Disadvantages
  - Costly
  - No indication of viability of organism or of susceptibility
- Still requires culture for confirmation, DST
- FDA-approved ONLY for respiratory secretions (sputum, bronchial)
  - smear +/- patients, ≤ 7 days therapy (Amplicor: smear + only)
- “Off-label” use
  - Physician education is important
**Nucleic Acid Amplification Testing for Respiratory Specimens**

- **Becoming standard of care … but do not test everyone**
  - Base testing on suspicion and communication with laboratory
  - Do not test smear positives when classic TB symptoms and history are present
  - Do test smear negatives when clinical suspicion of TB is high
- **CDC** – “Test at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered (i.e. Real suspects) but has not yet been established, and for whom the test result would alter case management and TB control activities”

* CDC Expert Panel on NAAT, MMWR 11/2008

**NAAT: Application and Interpretation**

<table>
<thead>
<tr>
<th>Sputum, Respiratory Secretions</th>
<th>NAA Positive</th>
<th>NAA Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dx of TB established; cult. still required</td>
<td>Consider clinical picture and repeat testing</td>
<td></td>
</tr>
<tr>
<td>Consider clinical picture and repeat testing</td>
<td>Unlikely that <em>Mtb</em> will be grown from sample (if controlled for inhibitor)</td>
<td></td>
</tr>
</tbody>
</table>

* MMWR (58:7-10), 2009

**Remove from Isolation?**

- **Airborne precautions can be discontinued when infectious TB disease is considered unlikely and either**
  - another diagnosis is made that explains the clinical syndrome,
  - the patient has three negative AFB sputum smear results, or
  - the patient has a sputum specimen that has a negative NAA test result and two additional sputum specimens that are AFB-smear negative.*

*CDC Expert Panel on NAAT, MMWR 11/2008

**Drug Susceptibility Testing (DST)**

- **Mandatory on all new patients**
  - 1st isolate from each site of disease; at 3mos if still cult pos.
  - Guides treatment decisions
    - Initially: for case and contacts
    - During treatment: determine reason for failure (emergence of resistance, absorption)
- **Accurate and timely reporting of results is essential**
  - Direct test; TAT 1 – 3 weeks
    - Smear positive cases; primary sample is tested
  - Indirect test; TAT 7+ weeks
    - Requires growth
  - Liquid media: 3 – 4 wk (can also do MIC for 1st line drugs)
- **Direct agar proportion method: Gold Standard**
  - Can test for multiple drugs, cheaply
  - Resolve agar/liquid media discrepancies

*CDC Expert Panel on NAAT, MMWR 11/2008
Susceptibility Testing of *M. tuberculosis* Complex

- Use rapid method (Broth-based)
  - Perform on all initial patient isolates
  - Test isolates from relapse or re-treatment cases; also if drug resistance suspected
- Test first-line drugs:
  - INH, Rif, EMB, PZA, SM* 
- Test second-line drugs and higher conc. INH, EMB, SM:
  - if R to rifampin or any 2 primary drugs
- Second-Line DST
  - Technically difficult (e.g., CS not recommended); not widely available
  - Cross-resistance
  - Methodologies not standardized – especially broth methods
  - Poor correlation with clinical response

**NCCLS Standard M24-A, 2003**

* M24A2 will drop SM, add AK and LQN

Discordance in DST

- Occurs between different labs, different methods, and within the same method
  - What do they mean?
  - Which is right?
- Many possible reasons …
  - Human
    - labeling, cross-contamination, …
  - Bacteria-specific
    - direct vs subculture, clumps, …
  - Methodology related
    - inoculation method, drug conc, media components, …

Laboratory Consortium

(4 public health labs and CDC)

- Discordance INH (low level)
  - Within lab (BT vs. AP) – 2.4%
  - Interlab (BT) – 6.0%
  - Interlab (AP) – 12%
- Discordance EMB
  - Within lab (BT vs. AP) – 6.1%
  - Interlab (BT) – 20.2%
  - Interlab (AP) – 8.7%
- Discordance PZA
  - Interlab (BT) – 4%

**Molecular DST**

- Molecular assays for INH, Rif most common
  - Detect polymorphisms associated with drug resistance
  - Performed on clinical specimens or culture isolates
  - Results available within 1-2 d
- In-house assays
  - Molecular beacons, pyrosequencing, RT-PCR
- Commercial assays
  - HAIN and INNO-LIPA line probe assays; Cepheid GeneXpert Rif
- Some Issues
  - Multiple mutations may confer resistance – not identified
  - Silent mutations – flagged but not really resistant
  - *None is FDA-approved* (9/2011)
Molecular Beacons

GeneXpert MTB/RIF™ Cepheid

- Closed, self-contained and automated platform
- rt-PCR-based amplification of MTb DNA
  - 131 CFU/mL clinical LOD
- Boehme NEJM 9/9/10: 1730 pulmonary TB suspects
  - Sensitivity: 551/561 sm-pos (98.2%); 124/171 sm-neg (72.5%)*
  - Specificity: 604/609 (99.2%)
  - Simultaneous detection of Rif resistance
    - 200/205 Rif-R (97%)
- Costly

*increased to 99% with repeat testing

Molecular Detection of Drug Resistance (MDDR) Service at CDC: Rationale

- Clinical/Program: available to providers
  - Make rapid confirmation of MDR TB available
  - Make laboratory testing data available to clinicians about second-line drug resistance in cases of Rif-resistant or MDR TB
- Development
  - Continuous correlation of molecular (genotyping) results and DST (phenotypic) results
  - Addition of new drugs and alleles
- Research
  - Determination of mechanisms of resistance

MDDR Service: Drugs and Genes for Panel

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP</td>
<td>rpoB</td>
</tr>
<tr>
<td>INH</td>
<td>inhA, katG</td>
</tr>
<tr>
<td>KAN</td>
<td>rrs, eis</td>
</tr>
<tr>
<td>AMK</td>
<td>rrs</td>
</tr>
<tr>
<td>CPM</td>
<td>rrs, tlyA</td>
</tr>
<tr>
<td>FQ</td>
<td>gyrA</td>
</tr>
<tr>
<td>PZA</td>
<td>pncA</td>
</tr>
<tr>
<td>EMB</td>
<td>embB</td>
</tr>
</tbody>
</table>
False-negative and False-positive results

- False-negative cultures
  - Improper collection/transport; overheating during transport/centrifugation; over-decontamination; media not inoculated correctly; clerical (labeling, transcribing, etc.)
- False-positive results
  - Another patient’s specimen or isolate; splashes; transfer on tools or aerosols during processing; contaminated reagents; AFB in water; clerical
- Clues
  - Increased number of sm +/- cultures detected by lab
  - Single positive culture among many submitted on patient
  - Delayed, scanty growth; multiple positive cultures on rack
  - Clinician: ... *No Way this is TB...*
- Resolution
  - Lab must have process in-place
  - Molecular testing often helpful

Serum Drug Level Monitoring

- Useful in selected circumstances
- Helps determine therapeutic concentrations
  - Allows adjustments for variable drug absorptions
- Documents adherence to treatment
- May reduce toxicities

Serum Drug Level Monitoring

- Aminoglycosides
  - To reduce toxicity, achieve therapeutic levels
  - In-house (Amikacin) vs send-out (Kanamycin)
- Ethambutol
  - Useful in renal insufficiency to reduce toxicity?
- Rifampin
  - To determine malabsorption (e.g. in severe HIV)
- Cycloserine
  - To determine therapeutic levels

The TB Laboratory: Challenges

- Declining case rates
  - Reduced competencies in low-incidence areas
  - *Level of service:* small labs “farming out” tests
- Shifting public health priorities
  - Reduced categorical funding for TB labs
  - Increased support for “crisis” responses (Anthrax, BT)
- Increasingly complex technologies
  - Capital investments
  - Training/educational needs of staff, users of services
- Demand for high-quality services
  - Budget issues
  - Public vs private
- Erosion of Public Health Laboratory’s key roles
## Lab vs Clinician: Two Sides of the Same Coin

- **Laboratory:** many specimens; few +
  - Low specificity tests:
    - e.g. smears; some NAA tests
    - many positives will be false
- **Clinician:** few patients are real TB suspects
  - Some will have positive specimens
  - Interpretation of a test result depends on suspicion of disease
  - Choice of management may depend on interpretation of low-specificity tests

### TABLE 3. Essential laboratory tests for tuberculosis control

<table>
<thead>
<tr>
<th>Test</th>
<th>Maximum turnaround time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy for acid-fast bacilli</td>
<td>≤24 hours from specimen collection or, if test is performed offline, ≤44 hours from receipt in laboratory; if latter, time from specimen collection to laboratory receipt should be ≤24 hours</td>
</tr>
<tr>
<td>Nucleic acid amplification assay</td>
<td>≤48 hours from date of specimen collection</td>
</tr>
<tr>
<td>Mycobacterial growth detection by culture</td>
<td>≤14 days from date of specimen collection</td>
</tr>
<tr>
<td>Identification of cultured mycobacteria</td>
<td>≤21 days from date of specimen collection</td>
</tr>
<tr>
<td>Drug susceptibility testing</td>
<td>≤30 days from date of specimen collection</td>
</tr>
<tr>
<td>Drug susceptibility testing of second-line drugs</td>
<td>≤4 weeks from date of request</td>
</tr>
</tbody>
</table>

(CDC: MMWR 54:11/14/2005)