The ABC’s of AFB’s
Laboratory Testing for Tuberculosis

Gary Budnick
Connecticut Department of Public Health
Mycobacteriology Laboratory
Overview

- Laboratory TAT Goals
- Case Study
  - Specimen Collection
  - Testing
- Contact Investigation
  - Genotyping
  - IGRA’s
- NAAT
# TAT Goals for the TB Laboratory

<table>
<thead>
<tr>
<th>Objective</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery of specimens to the laboratory</td>
<td>≤ 24 hrs of collection</td>
</tr>
<tr>
<td>AFB smear results reported to submitter</td>
<td>≤ 24 hrs of specimen receipt in lab</td>
</tr>
<tr>
<td>Identify growth as AFB ASAP</td>
<td>≤14 days of specimen collection</td>
</tr>
<tr>
<td>Isolates identified as MTB ASAP</td>
<td>≤21 days of specimen collection</td>
</tr>
<tr>
<td>DST results ASAP</td>
<td>≤28 days of specimen collection</td>
</tr>
<tr>
<td>NAA results on specimens ultimately TB culture (+)</td>
<td>≤48 hrs of specimen receipt in lab</td>
</tr>
</tbody>
</table>
Case Study

(details changed for presentation purposes)

- June 2006: Teenager immigrates to U.S.
- November 2009: started cough
- March 2010: presented to ED w/cough
  - Chest X-ray: Cavity in Left Upper Lobe
- Patient transferred to another hospital
  - CT Scan- Bilateral Cavities
- Specimens to commercial lab
  - Smears AFB(+)
Case Study

Sputums are Submitted to the State PH Lab for Smear and Culture

- Collected from 4/4/10 to 8/1/10

- Seventeen sputums are collected until the smear & culture are negative
Sputum Specimen Collection
The accuracy of laboratory testing is directly related to the quality of the specimen

- Proper collection technique prevents contamination of specimen
  - Any contaminants (other AFB & bacteria) introduced into the sputum decreases the chances of culturing out the TB
  - Cough directly into tube
- Use a sterile, single-use container (50-ml centrifuge tube)
- Patient identifier on tube
  - Lab will not test an unlabeled specimen
Sputum Specimen Collection

Cough deeply to produce a lower respiratory specimen

3 consecutive sputums collected in 8-24h intervals, one should be early morning (before eating, drinking or smoking)

Do not pool specimens

A good specimen should be approximately 3–5 ml, usually thick and mucoid. Clear saliva or nasal discharge is not suitable as a TB specimen
Package and Transport to Lab

- Packaging as per DOT/USPS regulations
- Complete & accurate test requisition
- Transport to lab ASAP
- If transport is delayed for more than one hour, refrigerate specimen
- Keep cold (do not freeze) during transport

CDC Goal: specimens received at laboratory within 24 hours of collection
New England Regional Data

Rapid Delivery of Specimens to Laboratory
Goal= 100% of Specimens Received within 24 hours of Collection

<table>
<thead>
<tr>
<th>Mean (2009)</th>
<th>% within 1-day</th>
<th>% within 2-days</th>
<th>% within 3-days</th>
</tr>
</thead>
<tbody>
<tr>
<td>New England</td>
<td>49.2</td>
<td>70.4</td>
<td>83.2</td>
</tr>
<tr>
<td>National</td>
<td>42.3</td>
<td>60.3</td>
<td>75.6</td>
</tr>
<tr>
<td>% difference</td>
<td>+6.9</td>
<td>+10.1</td>
<td>+7.6</td>
</tr>
</tbody>
</table>

Issues Affecting TAT
Method and Frequency of Transport
Coordination of specimen collection and delivery
Batching
Cost
State PH Labs Receive Cultures and Clinical Specimens for Testing

- Referred Cultures
  - AFB (+) Broths & Agar

- Clinical Specimens
  - Pulmonary most common
  - CTDPH (2009)
    - Sputum, Bronch Lavage, Bronch Wash & Brushes = 65.7%
Referred Cultures

BactiT/ALERT*
SEPTI-CHECK
LJ Agar Slant
7H10 Agar Slant
BACTEC 460*
SEPTI-CHECK Broth

*Automated Method
Processing and Culturing of Clinical Specimens

Digestion (NALC) Decontamination (NaOH) is lethal to all bacteria, AFB more resistant

Prepare & Stain Smears

Inoculate Broth & Agar Cultures:

Lowenstein-Jensen 7H10/7H11

MGIT Broth
Acid-Fast Staining of Clinical Specimens

Smears Read Daily

CDC Goal: Submitter receives AFB smear results within 24 h of receipt of specimen in lab
## Case Smear History

Patient hospitalized for approximately 2 weeks. Discharged to home isolation while smear positive

<table>
<thead>
<tr>
<th>Date Collected</th>
<th>Smear Result</th>
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<th>Smear Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/4/10</td>
<td>(+) Numerous</td>
<td>6/12/10</td>
<td>(+) Rare</td>
</tr>
<tr>
<td>4/11/10</td>
<td>(+) Numerous</td>
<td>6/18/10</td>
<td>Negative</td>
</tr>
<tr>
<td>4/21/10</td>
<td>(+) Numerous</td>
<td>6/27/10</td>
<td>Negative</td>
</tr>
<tr>
<td>5/1/10</td>
<td>(+) Few</td>
<td>7/8/10</td>
<td>Negative</td>
</tr>
<tr>
<td>5/8/10</td>
<td>(+) Rare</td>
<td>7/15/10</td>
<td>Negative</td>
</tr>
<tr>
<td>5/15/10</td>
<td>(+) Rare</td>
<td>7/22/10</td>
<td>Negative</td>
</tr>
<tr>
<td>5/22/10</td>
<td>Negative</td>
<td>7/28/10</td>
<td>Negative</td>
</tr>
<tr>
<td>6/5/10</td>
<td>Negative</td>
<td>8/1/10</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Acid-Fast Staining: Clinical Specimens

- Lab will report the “number” of AFB on AFB positive smears (rare, few, etc)
- You may see other smear reporting systems (e.g. CDC) 1+, 2+, 3+, 4+
- Fluorochrome stain most sensitive
  - Three AFB / smear is considered positive
- Non-Fluorochrome Stains
  - Kinyoun, Ziehl-Neelson,
What is an AFB?

- Acid-Fast Bacilli
- The bacilli stays “red” even after exposure to acid during staining
- TB & all Mycobacteria
- Other non-Mycobacteria e.g. Nocardia
# Sputum Collected 4/4/10

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<td>Positive (Numerous)</td>
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Culture (grow the AFB) in Clinical Specimens

LJ slants: 8 weeks @ 36 deg. C. w/CO₂

Automated system monitors growth

MGIT: Mycobacterial Growth Indicator Tube
Culture Progress (sputum collected 4/4/10)

- 4/10/10: Broth culture positive (AFB confirmed by smear)
- Submitter was notified of positive culture by phone and fax same day.
- Report: “Culture Positive for Acid-fast Bacilli”
- Not necessarily *M. tuberculosis*
Sputum Collected 4/4/10

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Is the Culture TB or Not TB?

Identification Tests

- **Gen-Probe Accuprobe**
  - primary ID method used by all NE PHLs
- **HPLC** (Connecticut)
  - AFB have unique mycolic acids used to ID them
- **Biochemical Testing** (all NE states)
- **Molecular** (CDC Reference lab)
  - DNA sequencing
What is a “Probe” Test?

There are basically two kinds of “probe” tests depending on what you are testing:

Amplified and Non-Amplified
Testing AFB (+) Cultures?
use Non-Amplified tests

- Gen-Probe Accuprobe
- MTbc, Mac, Mka, Mgo kits
- Tests for the presence of mycobacteria genetic “fingerprint”
- Sensitivity is based on how much AFB is in the culture
- Have to wait for growth
- Culture need not be pure
Testing Sputums Directly? Use an Amplified Test

• “Amplified” means copies of the TB are made which increases sensitivity
• MTD, NAA, NAAT, PCR
• MTD: MTbc
• Tests for genetic material (DNA or RNA)
• Patient should not have been treated
• For initial diagnosis, not to follow treatment
• Results within 24-48 h of specimen receipt
Identification

• 4/4/10: First sputum collected and received at the lab
• 4/10/10: Culture: Positive for *M. tuberculosis* complex by Gen-Probe Accuprobe
• TAT= 6 days

**CDC Goal:** Cultures identified within 21 days of specimen collection
What is *M. tuberculosis* complex

- A group of genetically related Mycobacteria
- *M. tuberculosis* – most commonly identified
- *M. bovis* - animals & humans
- *M. bovis* BCG - vaccine, bladder cancer therapy
- *M. africanum* - West Africa
- *M. canetti* - Africa, mode of transmission unknown
- *M. microti* – TB in voles
- Lab can confirm as *M. tuberculosis* if needed
Sputum Collected 4/4/10

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<tr>
<td>4/10/10</td>
<td>Gen-Probe Accuprobe MTBC</td>
<td>Positive for MTB complex</td>
</tr>
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</table>
Any Questions So Far?
Drug Susceptibility Testing (DST)

Goal: MTB DST results ≤ 28 days of specimen collection

BACTEC MGIT 960 Method (Most NE, VT uses VersaTREK)
• Set up first (ASAP after TB is identified)
• **Rapid** Broth Method (4-14 days)
• SIRE & PZA

“Confirmatory” Testing
• Set up if any resistance by MGIT 960
  • Secondary drugs also if Rif or any 2 drugs Resistance
• **21 Days Incubation**
• Agar Proportion Method “Gold standard”
BACTEC MGIT 960
Primary (First Line)
Anti-tuberculous Drugs

Rapid Method (4-14 days)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>1.0</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.1</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5.0</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>100</td>
</tr>
</tbody>
</table>
BACTEC MGIT DST Results

- Completed 4/29/10
  Isoniazid (INH) - Resistant
  Rifampin (RIF) - Resistant
  Steptomycin (STR) - Susceptible
  Ethambutol (ETH) - Susceptible
  Pyrazinamide (PZA) - Susceptible
  = Multidrug-Resistant TB

- TAT=25 days
  Goal: MTB DST results ≤ 28 days of specimen collection
What is Multidrug-Resistant TB?

A TB Isolate Resistant to Isoniazid and Rifampin
Agar Plate Method for primary confirmation and second line anti-tuberculous Drugs

“Gold Standard” method

Compare growth on drug and drug-free quadrants

Requires 21 days incubation
# Agar Plate Method (7H10)

## Primary and Secondary Drugs

<table>
<thead>
<tr>
<th>Primary Drug</th>
<th>Concentration (µg / ml)</th>
<th>Secondary Drug</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>0.2</td>
<td>Amikacin (Inj)</td>
<td>6.0</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>1.0</td>
<td>Ciprofloxacin (Flu)</td>
<td>2.0</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.0</td>
<td>Ethambutol</td>
<td>10</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5.0</td>
<td>Streptomycin</td>
<td>2.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0</td>
<td>Ethionamide</td>
<td>5.0</td>
</tr>
<tr>
<td>Kanamycin (Inj)</td>
<td></td>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td>p-Aminosalicylic Acid</td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Ofloxacin (Flu)</td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Capreomycin (Inj)</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Rifabutin</td>
<td></td>
<td></td>
<td>2.0</td>
</tr>
</tbody>
</table>
4/17/10- MGIT In Progress Report: Resistance Detected
4/18/10- Isolate Sent to CDC for MDDR Testing

• Molecular Detection of Drug Resistance
• Quick way to get a preliminary DST result
• CDC Program
• Looks at the the TB genes that cause resistance
• CDC Requirements
  • High risk of resistance or known Rif-R or high profile case
  • Mixed or Non-viable (won’t grow) cultures
• Limitations
  ▪ They don’t know all the genes that cause resistance in TB
CDC MDDR Results Received 4/21/10
= Multidrug-Resistant TB

- rpoB Mutation Found = **Rifampin Resistant**
- inhA - No Mutation
- katG - Mutation found = **Isoniazid Resistant**
- gyrA (fluoroquinolone) - No Mutation
- Rrs (Amikacin) - No Mutation
- Eis (Kanamycin - No Mutation
- Tlya (Capreomycin) - No Mutation
### Sputum Collected 4/4/10

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<td>4/10/10</td>
<td>Gen-Probe Accuprobe MTBC</td>
<td>Positive for MTB complex</td>
</tr>
<tr>
<td>4/21/10</td>
<td>CDC MDDR</td>
<td>Inh, Rif (Res)</td>
</tr>
<tr>
<td>4/29/10</td>
<td>BACTEC 960 DST</td>
<td>Inh (0.1), Rif (1.0) Resistant Str (1.0), Eth (5.0), PZA Sus</td>
</tr>
<tr>
<td>5/13/10</td>
<td>Agar Proportion 1&lt;sup&gt;st&lt;/sup&gt; &amp; 2&lt;sup&gt;nd&lt;/sup&gt; line</td>
<td>Inh (0.2, 1.0, 5.0) Resistant Rif (1.0) Resistant Eth (5.0) Resistant Strep (10.0) Resistant Rifabutin (2.0) Resistant Ethionamide (10.0) Resistant Others Susceptible</td>
</tr>
</tbody>
</table>
Why do the MGIT and APM DST Results Sometimes Disagree?

- Bacterial population (isolate vs. subculture)
- Differential growth rates
- Different inoculation amounts (size, clumps)
- Different methods or media
- Cross-contamination
- Transcription, labeling errors
- “Human error / lab error”

Beverly Metchock, DrPH, D(ABMM)
Mycobacteriology Laboratory Branch
CDC DTBE
Contact Investigation-Genotyping

- MTbc isolates forwarded to Michigan PHL
- Goal: send all MTbc case isolates
- Molecular fingerprinting patterns
- Compare patterns in state & nationally
- Uses:
  - Detect outbreaks earlier
  - Discover unsuspected relationships between cases
  - Identify false positives
- TB-GIMS (web-based tool)
Contact Investigation-IGRA’s

- Mother had TB when first immigrated
- Father treated for LTBI as contact to wife
- Patient was oldest of 5 children, including 3 week old infant, when diagnosed
- Quantiferon Testing:
  - 2/4 siblings positive (infant negative)
Interferon Gamma Release Assays (IGRA’s)

- Measures interferon gamma (INF-γ) released by WBC’s.
- Approved to measure immune response for both LTBI and TB infection resulting in active disease
- FDA approved tests:
  - QuantiFERON-TB Gold (QFT-G) 2005
  - Quantiferon-Gold (In-Tube) (QFT-GIT) 2007
  - T-SPOT.TB (T-Spot) 2008
- QFT-GIT: Connecticut & Massachusetts PHL
QuantiFERON Gold (In-Tube)
Best practices to optimize test results

- Collection & Handling Will Affect Results
  - Tubes at room temperature
  - Collect 1 ml (line on tube)
  - Vigorous shaking for 10 seconds
  - Store tubes upright
  - Do not refrigerate or freeze during storage or transport
  - Blood tubes must be incubated within 16 hours

- www.cellestis.com
Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* infection—United States, 2010. MMWR June 25, 2010/59(RR05); 1-25
Interferon Gamma Release Assays Recommendations

- Report both qualitative and quantitative results
- May use an IGRA in place of (but not in addition to) a TST in all situations in which CDC recommends a TST
- IGRA Preferred / TST acceptable
  - Low rates of return to have TST read
  - Received BCG vaccine or cancer therapy
- TST Preferred / IGRA acceptable
  - Children aged <5 y
Nucleic Acid Amplification (NAA) Test

- Make copies of TB DNA (if present)
- Specimen type depends on the method
- Does not distinguish live or dead AFB
- Sensitivity: **how frequently is NAA(+) when TB is in the specimen**
  - >95% for smear (+) TB patients
  - 55-75% of smear (-) / culture (+) patients
- Performance improves with increased clinical suspicion of TB
Amplified Mycobacterium Tuberculosis Direct (MTD)

- FDA-approved for smear (+) & smear (-)
- Not for test of cure or treatment monitoring
- Pulmonary Only
- None or <7d on drug therapy
- Approx. $60 / test cost (labor & reagents)
- NE PHL Availability
  - In-House: Connecticut, Maine, Massachusetts, Vermont
  - NH sends to Maine, RI sends to MA
Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

MMWR January 16, 2009/58 (1); 7-10
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Thank You