Importance of Laboratory & Clinical Monitoring

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Medical Evaluation for Active TB

- Medical history
- Physical examination
- Test for TB infection
- Chest radiograph
- Bacteriologic examination

Mycobacterial Examination

Mycobacterial examination has 6 stages:
1. Proper specimen collection
2. Examination of acid-fast bacilli (AFB) smears
3. Direct identification (NAAT-nucleic acid amplification test)
4. Specimen culturing and final identification
5. Drug susceptibility testing
6. TB genotyping

Specimen Sources

- Sputum (primary)
- Pulmonary aspiration (secondary)
- Gastric aspiration (less preferred, must reach the lab within 72 hours, must be neutralized to pH 6.0-8.0)
- Body fluids (CSF, pleural, peritoneal, etc)
- Tissues
- Blood
- Stool (special request)
- Other

Specimen Collection

Pulmonary Specimen (sputum)
- Early morning specimens = highest yield of AFB
- Collect at least three consecutive specimens at 8-24 hr intervals (at least 1 early morning specimen)
- Recommended volume for testing is 5-10 ml, less may compromise recovery of AFB
- Follow infection control precautions during specimen collection
- If patient cannot produce sputum by coughing, consider other methods: sputum induction, bronchoscopy, or gastric aspiration
- All persons suspected of TB disease should have sputum cultured

Specimen Collection

- Collect in sterile, leak proof containers
- Refrigerate specimen to reduce overgrowth of contaminating bacteria during transit to lab
- Deliver specimen to TB lab within 24 hrs
- Always include patient name on both test request form and the specimen container
Acid-fast Bacilli (AFB) smear

- Least sensitive of all AFB Tests (20-75%)
- Requires 10,000 AFB/ml to be positive
- Positive slide cannot determine AFB viability
- Positive slide does not determine whether TB or atypical mycobacteria (M. avium)
- Reported within 24 hours of receiving the specimen in the laboratory

Fluorescent AFB Smear Using Auramine-O Staining

- Very sensitive, takes minutes to read (read under lower magnification)
- Not all that is fluorescent is AFB (needs a careful eye)
- Can be confirmed with Ziehl-Neelson (ZN) smear
- Chemical fluorescence, not an immune stain or Direct Fluorescent Antibody

AFB Smear by ZN

- Usually takes about 15 minutes per slide
- May be used to confirm a positive fluorescent smear
- Used to confirm positive growth from slant and liquid media

AFB Smear ZN Interpretation

<table>
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<tr>
<th>AFB Seen (1000X)</th>
<th>Report</th>
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<tbody>
<tr>
<td>0</td>
<td>No AFB Seen</td>
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<tr>
<td>1-2 / 300F (3 sweeps)</td>
<td>Doubtful; repeat</td>
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<tr>
<td>1-9 / 100F</td>
<td>Rare (1+) AFB</td>
</tr>
<tr>
<td>1-9 / 10F</td>
<td>Few (2+) AFB</td>
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<tr>
<td>1-9 / F</td>
<td>Moderate (3+) AFB</td>
</tr>
<tr>
<td>&gt;9 / F</td>
<td>Numerous (4+) AFB</td>
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AFB Smear

AFB (shown in red) are tubercle bacilli

Direct Detection Using Nucleic Acid Amplification (NAA)

- NAA tests rapidly identify a specimen via DNA and RNA amplification
- Benefits may include
  - Earlier lab confirmation of TB disease
  - Earlier respiratory isolation and treatment initiation
  - Improved patient outcomes; interruption of transmission
- Perform at least 1 NAA test on each pulmonary TB suspect
- A single negative NAA test does not exclude TB
Nucleic Acid Amplification (NAA) Test

Amplified MTD by Genprobe is the only FDA approved method

New CDC Guidelines of Use of MTD (NAAT)

MMWR January 16, 2009

• “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.”

NAA: Molecular Detection of M. tb

• Rapid Test -24-48 hrs from positive / negative slide report
• AFB smear +: 97% sensitive/100% specific
• AFB smear -: 72% sensitive/99% specific
• Non-Bloody Pulmonary Specimens only
• M. celatum and M. terrae-like organisms may cross-react. However, M. celatum and M. terrae-like organisms are rare clinical isolates

AFB Culture Test

• More sensitive than AFB smear
• 10 AFB/ml can produce a positive result
• Culture may be AFB positive even if smear was reported negative for AFB
• Rapid broth testing – normally positive within 1-2 weeks. Requires 6 weeks to report culture as negative
• Positive culture result may be either Mycobacterium tuberculosis complex or atypical Mycobacterium

AFB Tests Performed on Growth in Mycobacteria Culture

• Genetic DNA Probe Identification (not amplified)
• HPLC (high performance liquid chromatography) Identification
• Biochemical Identification Confirmation
• Drug Susceptibility
• DNA Genotype
• MDDR (molecular detection of drug resistance) (CDC)
Susceptibility Testing of *M. tuberculosis*

**When to test**
- All new (initial) *M. tb* isolates
- Suspected new drug resistance
- Repeat after 90 days if specimens continue to produce *M. tb*
- Relapse or failed therapy

**Primary Anti-TB drugs**
- Isoniazid
- Rifampin
- Ethambutol
- Streptomycin
- Pyrazinamide

**Secondary Anti-TB Drugs**
- Fluoroquinolone (ciprofloxacin, levofloxacin or moxifloxacin)
- Ethionamide
- Cycloserine
- Capreomycin
- Amikacin
- Kanamycin
- Streptomycin
- PAS

**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

**CDC – Molecular Detection of TB Drug Resistance (MDDR)**
- Rapid testing for DNA sequences associated with 1<sup>st</sup> and 2<sup>nd</sup> line drug resistance
- NAAT (+) sputum specimens or culture isolates
- 3-4 day turn-around-time
- Must meet the following criteria:
  - Known Rifampin resistance
  - Known MDR
  - High risk of Rifampin resistance or MDR-TB (e.g. previous TB, MDR-TB contact, foreign born)
  - High profile patient (e.g. daycare worker, nurse)
  - Mixed or non-viable culture
  - Adverse reaction (e.g. RIF allergy)

**CDC MDDR**
- **First-line** MDDR to detect MDR-TB
  - *rpoB* (Rifampin)
  - *inhA* and *katG* (Isoniazid)
- **Second-line** MDDR to detect XDR-TB
  - *gyrA* (Fluoroquinolones)
  - *rrs* (Kanamycin, Amikacin, Capreomycin)
  - *eis* (Kanamycin)
  - *tlyA* (Capreomycin)
  - *pncA* (Pyrazinamide)
  - *embE* (Ethambutol)
- Used alone, MDDR and DST are imperfect, used together, the accuracy of the detection of drug resistance can be improved
TB DNA Genotyping
Universally Offered by CDC
• “Fingerprint” of each isolate
• Michigan Department of Community Health – Eastern States
• California Department of Public Health – Western States

Genotyping (cont.)
Used with traditional epi investigations, genotyping has
• Confirmed/detected transmission
• Identified risk factors for recent infection
• Demonstrated re-infection with different strains
• Identified weaknesses in conventional contact investigations
• Documented lab cross-contamination
• Identified outbreaks not previously recognized

Epidemic Curve of Investigation of a Multistate TB Outbreak

3 Examples of TB suspects
• Each presents on October 1, 2012
  • EASY #1 case, with smear + pulmonary TB
  • MEDIUM #2 case, with smear negative pulmonary TB
  • DIFFICULT #3 case, with suspected MDR-TB

SAMPLE CASES....
Case #1

Figure Legend:
Admission chest radiograph showing bilateral lung infiltrates, with prominence in the right upper lobe and lingula of the left lung.
#2 case

**December 2012**

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**Case #2**

57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³

#2 case

**October 2012**

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**Case #3**

57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³

#2 case

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#3 case MDR suspect

**Date:** October 2012

**10/2/2012**

**#3 case MDR suspect**

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**Notes:**

- 8 PPD or IGRA
- 9 SPUTUM COLLECTED 1ST
- 10 SMEAR POSITIVE PPD READ 15 mm
- 11 SMEAR POSITIVE + SMEAR POSITIVE
- 12 MDDR POSITIVE

**Reference:**

- [http://www.cdc.gov/tb/topic/Laboratory/mddr.htm](http://www.cdc.gov/tb/topic/Laboratory/mddr.htm)

**MDCH Lab:** April 24, 2009

- **INH:** R
- **Rifampin:** R
- **FZX:** R
- **Ethambutol:** R
- **Ciprofloxacin:** R
- **Ofloxacin:** R
- **Ethionamide:** R
- **Streptomycin:** S
- **Kanamycin:** S
- **Aminosidine:** S
- **Capreomycin:** S
- **Cycloserine:** S
- **PAS:** S

**Value of Mycobacteriology Lab Test Results**

- Confirm evidence of AFB infection (TB)
- Support TB clinical diagnosis
- Clarify progress of TB infection/disease
- Determine infectivity of patient
- Determine appropriate drug regime
- Monitor for development of resistance
- Confirm treatment success
- Determine relationship to other cases
3-Fold Interactions for TB Care...