Public Health Mycobacteriology (TB) Laboratory Testing Services

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Overview

- Specimen Collection & Submission
- Laboratory Facilities & Safety
- Test Methods and TAT Goals
- Clinical Specimen-Processing, Smear, Culture
- Acid-Fast Bacilli (AFB) Identification Methods
- Drug Susceptibility Testing
- IGRA’s and QuantiFeron
- Nucleic Acid Amplification Tests (NAATs)
Clinical Specimen Collection and Submission

The accuracy of laboratory testing is directly related to the quality of the specimen

Use aseptic technique to collect specimen

- Clean, sterile, single-use containers
- Proper technique prevents contamination with environmental AFB & non-AFB
- Avoid swabs

Packaging & Shipping

- Packaging for DOT regulations & safety of carrier & receiver
- Complete & accurate test requisition
- Specimen type determines processing method
- Refrigerate if shipping is delayed (minimize overgrowth) except blood

Transport to laboratory ASAP

CDC Goal: specimens received at laboratory within 24 hours of collection
Mycobacterial infections can be localized or systemic therefore a variety of samples are appropriate for laboratory analysis

The CTDPH TB Laboratory analyzes approximately 3500 clinical specimens annually

**Pulmonary**
Sputum, Bronchoalveolar Lavage Fluids, Bronchial Washings & Brushes

**Extra-Pulmonary**
Fluids (Synovial, Pleural, Peritoneal, Paracentesis, Pericardial, Ascites, CAP dialysis, Laryngeal Swabs & Aspirates, Stool, Urine, Tissue, Lymph Nodes, Bone, Biopsies, etc. etc. etc.
Blood (whole blood in SPS or Heparin), Bone Marrow, CSF (2-3 ml)
Gastric wash/lavage (Na Carbonate to neutralize if <1 hr)

Sterile vs. non-sterile specimens processed differently
**Biosafety Level-3 (BSL-3) Facilities and Practices**

- **Biosafety Level = Infectious agent + (lab practices / facilities / safety equipment)**
- **BSL-3**
  - Laboratory personnel trained in handling pathogenic and potentially lethal agents
  - Controlled access
  - Special engineering and design features. Unidirectional airflow
  - All procedures involving the manipulation of infectious materials must be conducted within Biological Safety Cabinets (BSCs)
  - Personal Protective Devices: solid-front gowns, gloves, N-95 or HEPA-filtered respirator
  - Laboratory personnel trained in handling pathogenic and potentially lethal agents
  - Annual TST or IGRA
CLASS II Type A Biological Safety Cabinet (BSC)

- Inward flow to protect personnel
- High Efficiency Particulate (HEPA)-filtered downward airflow to protect specimens
- HEPA-filtered exhaust for environmental protection
BSC for Probes and Susceptibility Testing

CO\textsubscript{2} Incubators
Clinical specimens reach the laboratory within 24 h of collection
Submitter receives AFB smear results within 24 h of receipt of specimen in lab
Culture positive ≤ 14 days of specimen collection
Isolates identified as TB ≤ 21 days of specimen collection
MTB DST results ≤ 28 days of specimen collection
NAAT results on ultimately TB culture (+) specimens ≤ 48 h of specimen receipt in lab
Processing of Primary Clinical Specimens

Digestion & Decontamination

Prepare & Stain Smears

Inoculate Culture:

Lowenstein-Jensen MGIT Broth
Acid-Fast Staining: Clinical Specimens

Smears Read Daily

Auramine-Rhodamine Fluorescent Staining

<table>
<thead>
<tr>
<th>Number of AFB in 1x2” smear</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No AFB found</td>
</tr>
<tr>
<td>1-2 in entire smear</td>
<td>Repeat</td>
</tr>
<tr>
<td>3-9 in entire smear</td>
<td>Rare</td>
</tr>
<tr>
<td>≥ 10 in entire smear</td>
<td>Few</td>
</tr>
<tr>
<td>≥ 1 per 1000 x field</td>
<td>Numerous</td>
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</table>
Acid-Fast Staining: Clinical Specimens

- “Quantitate (+) smear results
- Other smear reporting schemes (CDC) report 1+, 2+, 3+, 4+.
- A-R Fluorochrome staining method most sensitive. Three AFB per smear (2-cm²) is reportable as positive
- Zieihl-Neelson, Kinyoun’s staining
Why in some cases is the smear positive and culture negative or smear negative/culture positive?

- The AFB may be present in the specimen but may be non-culturable (non-viable or, dead) or “injured” due to drug treatment so they may not grow as well.

- Culture is more sensitive than smear. The sensitivity of the smear ranges from 22-78% (specificity=99.3-99.7%). A positive smear indicates at least 10,000 AFB / ml specimen.
Culturing of Primary Clinical Specimens

LJ slants: 8 weeks @ 36 deg. C. w/CO₂

Automated system monitors growth

MGIT: Mycobacterial Growth Indicator Tube
Culturing Primary Clinical Specimens

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

*Automated Method
Cultures incubated and read weekly for up to 8 weeks
7H10 & LJ Agar
Dubos-Tween Broth

“Acid-fast” microorganisms isolated

Cultures proceed to identification methods
AFB Culturing Issues

Why do some Mycobacteria cultures take longer to grow than others, even from the same patient?

- There are currently >120 classified species of mycobacteria.
- The number of organisms in the specimen (the bacterial burden), the “condition” of the AFB and the growth conditions will affect the growth rate and culturability.
- Mixed cultures
- Generally, most mycobacteria, including TB, are “slow growers” (14-21 days). “Rapid growers” (*M. fortuitum* complex) grow within 7 days; some take >21 days (*M. xenopi*).
Mycobacteria Identification Methods: Probes

- Gen-Probe Accuprobe (MTBC, MAC, MGO, MKA)
- “Rapid” test: approx. 2 hours to perform
- Tests for the specific mycobacteria in the culture by looking for its DNA “footprint”
- Have to wait for bug to grow
- Culture need not be pure
- Cannot be used on clinical specimens
- “Direct” probe test: only amount present in culture.
- MTbc can be speciated by additional testing
- CTDPH lab tests twice weekly (Tuesday & Friday) and on request
What is the Mycobacterium tuberculosis complex?

- A group of closely related Mycobacteria (16S rDNA)
- *M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, M. canetti, and M. microti*
  - *M. bovis*-animals & human, zoonotic TB, 3rd world
  - *M. bovis BCG*-attenuated TB vaccine, bladder cancer therapy-may disseminate in immunocompromised (HIV)
  - *M. africanum*-West Africa, OK if immunocompetent
  - *M. canetti*-Africa, natural reservoir, host range, and mode of transmission unknown
  - *M. microti*-naturally acquired generalized TB in voles
- *M. tuberculosis* most common
Can a probe test be negative for MTBC and still contain TB and ultimately be culture positive (i.e. false-negative probe result)?

Yes. If the number of organisms is below the detectable amount, the test will be negative. Culture must show sufficient growth to be a “true-positive”

The CTDPH laboratory has seen negative MAC probe results with positive HPLC.

Why can’t Accuprobe testing be done on sputum directly?
The test has been FDA-cleared for testing with cultures only.
HPLC
(High Performance Liquid Chromatography)

- Mycobacteria have mycolic acids in their cell wall (makes them “acid-fast”)
- Mycobacterium species have unique types and amounts of mycolic acids that can be used to identify them to species level.
- CTDPH laboratory uses the MIDI Sherlock system
  - HPLC w/analysis software and pattern libraries
MIDI SHERLOCK
MYCOBACTERIA ID
SYSTEM

Distinguishes *M. bovis* BCG from the TB complex.

- Rapid extract-to-ID in 15 minutes.
- Easy-to-use automated analysis and naming.
- Analyze up to 100 samples a day.
- Mycobacteria are killed during sample preparation.
- Environmentally-friendly methanol and isopropanol solvents used.
HPLC Chromatograms

M. tuberculosis

M. szulgai
Identification Issues

Goal: Cultures identified within 21 days of specimen collection.

Why does are MTBC results reported prior to NTM found in the same culture?

Accuprobe results will only identify the target tested for (MTBC, MAC, etc.) whereas subsequent subculture and HPLC will show NTM. NTM usually found in lower numbers esp. if from contamination
Drug Susceptibility Testing (DST)

Goal: MTB DST results ≤ 28 days of specimen collection

BACTEC MGIT 960 Method
- SIRE & PZA automatically on initial patient & requests
- Set up ASAP after TB is identified
- Results phoned/faxed

“Confirmatory” Testing
- Agar Proportion Method “Gold standard”
- Primary drugs when any resistance on BACTEC
- Secondary Drugs also when Resistant to Rif or any two primary drugs
BACTEC MGIT 960
Primary (First Line)
Anti-tuberculous Drugs

Rapid Method (4-14 days)
## BACTEC MGIT 960 Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>1.0</td>
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<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>
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<tr>
<td>Pyrazinamide</td>
<td>100</td>
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</table>
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
<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</td>
<td>6.0</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>1.0</td>
<td>Ciprofloxacin</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>
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<tr>
<td></td>
<td></td>
<td>Kanamycin</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Aminosalicylic Acid</td>
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<tr>
<td></td>
<td></td>
<td>Ofloxacin</td>
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<tr>
<td></td>
<td></td>
<td>Capreomycin</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycloserine</td>
<td>25</td>
</tr>
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</table>
What does it mean when a TB isolate is Resistant to a drug? When >1% of the TB organisms in a population are resistant to a given drug. Clinical success is less likely.

What determines the drug concentrations used in DST’s? The lowest concentration that inhibits a “wild-type” strain of Mtb (has never been exposed to the drug), while at the same time does not inhibit strains that have been isolated from patients who are not responding to therapy and are considered resistant. This is the concentration that best discriminates between S and R strains = critical concentration. Critical concentrations are “comparable” between methods but they may differ among test systems. This causes confusion.
Reasons for Discordant DST Results

- Bacterial population (isolate vs. subculture)
- Differential growth kinetics
- Different inoculation methods (size, clumps)
- Different methods or media
- Cross-contamination
- Transcription, labeling errors
- “Human error / lab error”
- The “bug” - the MIC is close to the critical concentration. Reproducibility poor
Interferon-Gamma Release Assays (IGRA’s)

- FDA-Approved Methods
  - QuantiFERON Gold
  - T-SPOT-TB
- If patient is infected with TB, their white blood cells will release IFN-gamma in response to contact with the TB antigens.
- More sensitive than TST (particularly in people with HIV and suppressed immune responses)
- More specific to TB: No FP because of BCG vaccination boosting effect from serial testing TST
QuantiFERON Gold (IT)

- 3 specialized blood collection tubes
- Must be incubated within 16 h. of collection.
- Test results in 2-3 h.
- Results:
  - (+) = *M. tuberculosis* infection likely
  - (-) = *M. tuberculosis* infection NOT likely
  - Indeterminate
- Each QFT-G result and its interpretation should be considered in conjunction with other epidemiological, historical, physical, and diagnostic findings.
Nucleic Acid Amplification Tests (NAAT’s)

- Works by making copies of the TB DNA (if present) and then detecting this DNA “footprint”
- Specimen type depends on the method
- Do not distinguish live or dead AFB
- TAT 24-48 h
- Sensitivity: how frequently is NAAT(+) 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
NAAT Availability

- FDA-cleared kits for respiratory specimens
  - Amplified Mycobacterium tuberculosis Direct (MTD): GEN-PROBE, Inc.
  - Amplicor M.tuberculosis (MTB): Roche Diagnostics
- Commercial tests are available outside the U.S.
- Home brew tests
- Off-label use
Amplified Mycobacterium tuberculosis Direct (MTD)

- FDA-approved for smear (+) & smear (-)
- Transcription Mediated Amplification
- Assay time 2.5 to 3 h
- Not for test of cure or treatment monitoring
- Sputum, Bronchials, Tracheal aspirates
- No or <7d drug therapy or none in 12 mo.
- Approx. $60 / test cost (labor & reagents)
Nucleic Acid Amplification Tests Guidelines

Notices to Readers: Nucleic Acid Amplification Tests for Tuberculosis MMWR Nov 1, 1996/45 (43); 950-952

- Potential uses of NAATs & interim guidelines
- Current NAATs (MTD) and FDA-approved uses
- Off-Label Uses
- Limitations & Cautions - NAATs do not replace any previously recommended tests (eg. culture).
- Conclusions: decisions on when and how to use NAA tests for TB diagnosis should be individualized, must be interpreted in clinical context, and may differ for PH and individual clinical decisions
Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

MMWR January 16, 2009/58 (1); 7-10
NAAT Recommendation

- Perform NAAT on at least one respiratory specimen if:
  - Signs and symptoms of pulmonary TB
  - TB diagnosis is being considered, not yet established
  - Test results would alter case management or TB control activities
- Standard specimen collection and process for smear & culture, lab creates volume to perform NAAT
- Perform NAAT on first patient diagnostic specimen
- Follow manufacturer’s instructions
- Interpret NAAT results in correlation with AFB smear, growth-dependent methods & clinical parameters
Algorithm is based on degree of clinical suspicion of TB

- Always interpret lab results on the basis of the clinical situation
  - Low Suspicion: two positive results are needed for decision
  - High or Intermediate Suspicion: One positive result needed for decision
NAAT Result Interpretation: NAAT (+)

- First Specimen
  - NAAT(+) / Smear (+)
    - Patient Presumed to have TB
      - No additional NAAT testing needed
      - Begin treatment pending culture results

  - First Specimen
    - NAAT (+) / Smear (-)
      - Clinical judgement on whether to treat pending culture results
      - Test additional specimen(s) to confirm NAAT (+) (Increases PPV)

- If ≥ 2 follow-up specimens NAAT(+):
  - Patient Presumed to have TB
  - Begin treatment pending culture results
NAAT Result Interpretation: NAAT (-)

First Specimen
NAAT (-) / Smear (+)

Test For Inhibitors

Inhibitors Detected
NAAT no help
Clinical judgment on whether to treat pending culture results
Additional Spec’s can be tested

Follow-up specimens NAAT(-) / Smear (+)
Presume Patient infected with NTM

Inhibitors Not Detected
Clinical judgment on whether to treat pending culture results
Should test 1-3 addit. specimens

First Specimen
NAAT (-) / Smear (-)*

Clinical judgment on whether to treat pending culture results
Additional spec’s can be tested

IF NAAT(-) / Smear (-), patient not infectious

*NAAT sensitivity is too low in NAAT (-) / Smear (-) Patients to exclude TB diagnosis
ADVANTAGES OF NAATs

- More rapid diagnosis
- Initiation of earlier treatment
- Faster Reporting to TB Programs
- Fewer Transmissions
- Cost savings for patient isolation

DISADVANTAGES OF NAATs

- No automation
- No self-contained formats
- Requires technical expertise
- Meticulously clean lab environment
- Sensitivity of NAAT cannot be expected to approach that of smear (+)
QUESTIONS