Tuberculosis Testing and Laboratory Updates

Arizona State Public Health Laboratory

Stephanie Kreis
Arizona Department of Health Services
Bureau of State Laboratory Services
Bacteriology/Mycobacteriology/Parasitology
kreiss@azdhs.gov
Overview

• Description of laboratory services at Arizona State Laboratory (ASL)
• Specimen collection and transport
• Testing workflow
• Tests available
Arizona State Laboratory

- Diagnostic and Reference laboratory
- Hours of Operation: Monday – Friday, 8:00am -5:00pm
- AZDHS Website http://www.azdhs.gov/lab/micro/index.htm
  – Guide to Laboratory Services
  – Laboratory Submission form
Arizona State Laboratory TB Services

• Processing of Diagnostic Specimens for AFB smear and culture
• AFB Microscopy
• AFB Culture and Identification
• Rapid testing: i.e. NAAT
• TB Drug Susceptibility testing
  – Broth method (rapid)
  – Agar Proportion method
Specimen Collection

- Specimen collection kits and mailing containers are provided by ASL
  - Contact by mail, phone, or fax
    - Arizona Department of Health Services
      Bureau of State Laboratory Services
      ATTN: Receiving Section
      250 N. 17th Ave
      Phoenix, AZ 85007
      Fax: (602) 542-0760
      Phone: (602) 542-1190
Example of Collection Kit Supplied by ASL

Inner metal screw capped container, outer screw capped cardboard container, place submission form around the outside of the inner metal container. **Label the specimen collection container with patient name and collection date.**
Specimen Collection

• Specimen collection containers requirements
  – Sterile
  – Leak-proof
  – Disposable
  – Non-breakable
  – Appropriately labeled!!!

• Laboratory Submission Form
Specimen Collection

• Quality specimens are vital for lab diagnosis of TB
• Collect aseptically, or bypass contamination as much as possible
• Avoid contamination with tap water (NTM may be present)
• Collect prior to therapy if possible
• No swabs, fixatives, preservatives
Laboratory Submission Form

Bureau of Laboratory Services
250 N. 17th Avenue Phoenix, Arizona 85007-3231
Tel: (602) 542-1188  Fax: (602) 364-0758
Victor Waddell, Ph.D., Bureau Chief

For Department Use Only

PATIENT INFORMATION

<table>
<thead>
<tr>
<th>Last name:</th>
<th>First name:</th>
<th>MI:</th>
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<tr>
<td>DOB (MM/DD/YYYY):</td>
<td>Age:</td>
<td>Sex: [M ☐ F ☐ T ☐]</td>
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<tr>
<td>Street address:</td>
<td>City:</td>
<td>State:</td>
</tr>
<tr>
<td>Ethnicity: Hispanic ☐ Yes ☐ No ☐</td>
<td>Race: [White ☐ African American ☐ Asian ☐ American Indian/Alaska Native ☐ Other ☐]</td>
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SUBMITTING AGENCY INFORMATION

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<tr>
<th>Agency name:</th>
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<td>Contact name:</td>
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<tr>
<td>Zip:</td>
<td>County: select one</td>
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<td>Tel:</td>
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ORDERING PROVIDER INFORMATION

<table>
<thead>
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SPECIMEN INFORMATION & TYPE

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<tr>
<td>Outbreak name:</td>
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# Laboratory Submission Form

<table>
<thead>
<tr>
<th>VIROLOGY</th>
<th>BACTERIOLOGY</th>
<th>SEROLOGY</th>
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<tbody>
<tr>
<td>CMV Culture</td>
<td>*Bordetella pertussis</td>
<td>*Borrelia burgdorferi EIA (Lyme)</td>
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<tr>
<td>Enterovirus Culture</td>
<td>Campylobacter spp.</td>
<td>*Borrelia Tube Ag.</td>
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<tr>
<td>Herpes Culture</td>
<td>Clostridium botulinum toxin</td>
<td>Coccidioides Serology Panel</td>
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<tr>
<td>Influenza</td>
<td>Corynebacterium diphtheriae</td>
<td>IDTP, IDCF</td>
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<tr>
<td>Norovirus PCR</td>
<td>Enteric culture</td>
<td>Dengue IgG EIA</td>
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<tr>
<td>Reference Virus Culture</td>
<td>Escherichia coli / Shigatoxin</td>
<td>Diagnostic Hepatitis Panel</td>
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<tr>
<td>Respiratory Virus Culture</td>
<td>Haemophilus influenza</td>
<td>EIA</td>
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<tr>
<td>Other</td>
<td>Legionella spp.</td>
<td>HAV IgM</td>
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<tr>
<td></td>
<td>Leptospira spp.</td>
<td>Francisella tularensis Tube Ag.</td>
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<td></td>
<td>Neisseria gonorrhoeae</td>
<td>*Francisella tularensis</td>
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<td>Neisseria meningitidis</td>
<td>*Hantavirus IgM EIA</td>
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<td>Salmonella spp.</td>
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<td></td>
<td>Shigella spp.</td>
<td>Hepatitis Anti-HAV IgM</td>
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<td>Vibrio</td>
<td>Hepatitis Anti-Core IgM</td>
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<td>VISA/VRSA</td>
<td>Hepatitis Anti-HCV</td>
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<td>Hepatitis HBsAG</td>
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<td>**Please refer to the Guide to Laboratory</td>
<td>*Measles IgM EIA</td>
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<td>Services: Microbiology, Section 8 for the</td>
<td>Mumps IgM EIA</td>
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<td></td>
<td>definition of select agents and the testing</td>
<td>Rickettsial Panel IFA</td>
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<td>Rickettsial Q Fever</td>
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<td><strong>Select AGENTS</strong></td>
<td>Rickettsial Spotted Fever</td>
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<td>Avian Influenza H5N1</td>
<td>Rickettsial Typhus Fever</td>
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<td>*Burkholderia spp.</td>
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<td>SARS</td>
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<td></td>
<td>*Yersinia pestis</td>
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<td></td>
<td>**Parasitology†</td>
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<tr>
<td></td>
<td>Arthropod ID</td>
<td>Nucleic Acid Amplification</td>
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<td>Blood/Tissue</td>
<td>Susceptibility</td>
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<td>Intestinal</td>
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<td>For malaria testing please collect patient</td>
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<td>travel history</td>
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<td>**Mycobacteriology†</td>
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<tr>
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<td>Culture</td>
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<td>ID (Referred Culture)</td>
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<td>*Nucleic Acid Amplification</td>
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<tr>
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<td>Smear</td>
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<tr>
<td></td>
<td>Smear</td>
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<tr>
<td></td>
<td>Other:</td>
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</table>

Substituting Lab Findings or Preliminary ID: ________________

* Prior notification is required for: Bacillus anthracis, Bordetella pertussis, Brucella spp., Clostridium botulinum toxin, Corynebacterium diphtheriae, emerging or exotic diseases, Francisella tularensis, Hantavirus, Borrelia burgdorferi (Lyme), Measles, Mycobacteria NAA, Rubella, or Yersinia pestis testing. CALL: (602) 364-3676

ALL FIELDS HIGHLIGHTED IN YELLOW ARE REQUIRED FOR SPECIMEN PROCESSING. IN ADDITION, AT LEAST ONE TEST MUST BE REQUESTED.

*Patient address and telephone number are required, when available, per R9-5-204(B3) [http://www.azdhs.gov/lab/microindex.htm](http://www.azdhs.gov/lab/microindex.htm) Updated 10/20/2010*
Laboratory Submission Form

- Complete all highlighted (required) fields
AFB Specimen Transport

- Transport in as short as a time as possible to avoid overgrowth of contaminating bacteria
- Specimens that cannot be transported to the lab immediately should be refrigerated
- Reference the Guide to Laboratory Services
- CDC recommendation:
  - Specimen received at laboratory within 24 hours of specimen collection.
Typical Specimen Workflow at ASL

Specimen Arrives at State Lab

AFB Smear
- Positive
  - NAAT
- Negative

AFB Culture
- Positive
  - Identification
  - Drug Susceptibility (MTB)
- Negative
AFB Smear Microscopy

- Quick and inexpensive
- Results should be available within 24 hours of receipt of specimen.
- Low sensitivity, 50-70% for pulmonary TB
- Not specific for MTBC
- Does not distinguish between live and dead bacilli
AFB Culture

• More sensitive than AFB smear
• Current recommendations are to use at least two types of media to maximize the recovery of mycobacterium
• Cultures monitored 6-8 weeks.
AFB Culture

• Liquid media typically provides more rapid results
• Combination of solid and liquid media maximizes recovery and provides an opportunity to look at colony morphology
Nucleic Acid Amplification Tests for Direct Detection of MTBC

- FDA-approved for use with respiratory specimens
- Non-FDA approved tests (RUO or not available in U.S.)
- Laboratory developed tests or LDT (e.g., DNA sequencing, Loop-mediated isothermal amplification [LAMP], and real-time PCR assays including molecular beacons)
Nucleic Acid Amplification Tests

- NAAT can detect MTBC genetic material directly from specimen within hours.
- Does not distinguish live and dead bacilli
- Results:
  - *Mycobacterium tuberculosis* complex detected
  - *Mycobacterium tuberculosis* complex not detected
    - Negative result does not necessarily mean absence of MTBC
      - Inhibition of amplification
      - Target below limit of detection
Nucleic Acid Amplification Tests

- Recommended Turnaround time: 24-48 hours from specimen receipt
- Most can be performed from smear positive or smear negative specimens
  - Sensitivity decreases for smear negative specimens
- Does not replace the need for culture confirmation. Culture still needed for conventional drug susceptibility testing and genotyping.
Updated Guidelines for the Use of NAAT in the Diagnosis of TB

• “NAAT 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.” MMWR, 2009, 58:7-10

• State TB control program and ASL collaborated to determine criteria for testing.
ASL’s NAAT Algorithm

Respiratory Specimen

- Initial Smear +
  - NAAT performed

- Smear -
  - NAAT performed upon approval
How to get approval for NAAT?

• Contact State TB control program
• Approval will be based on whether the patient meets the following criteria
  – Have a cavitary lesion seen on CXR
  – Are in a long-term care facility
  – Are HIV positive
  – Are immunocompromised
  – Are on dialysis, or
  – If it will make a difference in the treatment/isolation of the patient
TB Drug Susceptibility Testing

• Broth Systems
  – Rapid results
  – Testing done from culture
  – FDA approved for first line TB drugs
    • Isoniazid, Rifampin, Ethambutol, PZA
    • Streptomycin
  – Recommended on all initial cases of MTBC
  – Should be repeated if there is clinical evidence of failure to respond to treatment or if cultures fail to convert to negative after 3 months of treatment.
Proportion Agar Drug Susceptibility Testing

- Proportion Agar Method
  - “Gold Standard” method used in the United States for several decades.
  - Equal quantities of several dilutions of a standard inoculum onto a agar based medium with and without drug.
  - Number of CFU growing on drug containing quadrants are compared to those on the drug free medium and expressed as a percentage.
  - Turnaround time: 21-28 days.

- If drug resistance is suspected contact ASL or State TB control program.
Proportion Agar Drug Susceptibility Testing
Molecular Detection of Drug Resistance

• Laboratory developed tests (LDT)
• Non-FDA approved tests (Research Use Only [RUO] or not available in U.S.)
Molecular Detection of Drug Resistance

- PCR-based DNA sequencing for drug resistance
- Criteria for accepting specimens
  - High risk patients (RIF-R, MDR-TB)
  - High profile patients
  - Known RIF-R
  - Mixed or non-viable cultures
How to request MDDR services?

- Contact State TB control program to make a request
- ASL will contact the CDC for approval to send the specimen
- ASL will arrange to ship the specimen to the CDC
- Preliminary report issued with molecular results
- Final report issued upon completion of agar proportion drug susceptibility testing
CDC Molecular Detection of Drug Resistance Service

- Drugs and Genes offered with MDDR service (CDC)

<table>
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<tr>
<th>Drug</th>
<th>Gene</th>
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<tbody>
<tr>
<td>RIF</td>
<td><em>rpoB</em></td>
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<tr>
<td>INH</td>
<td><em>inhA, katG</em></td>
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<tr>
<td>FQ</td>
<td><em>gyrA</em></td>
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<td>KAN</td>
<td><em>rrs, eis</em></td>
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<td><em>rrs</em></td>
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<td>CAP</td>
<td><em>rrs, tlya</em></td>
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<tr>
<td>EMB</td>
<td><em>embB</em></td>
</tr>
<tr>
<td>PZA</td>
<td><em>pncA</em></td>
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National TB Genotyping Program

• Began in January, 2004
• Goal: Genotype at least one isolate for every culture-positive case of TB
• Arizona State Lab actively participates in program by routinely submitting isolates to the assigned genotyping laboratory.
National TB Genotyping Program

• Results are uploaded to a national database.

• Results are used to
  – Confirm suspected links
  – Detect unsuspected transmission
  – Detect false positive cultures
Questions???