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

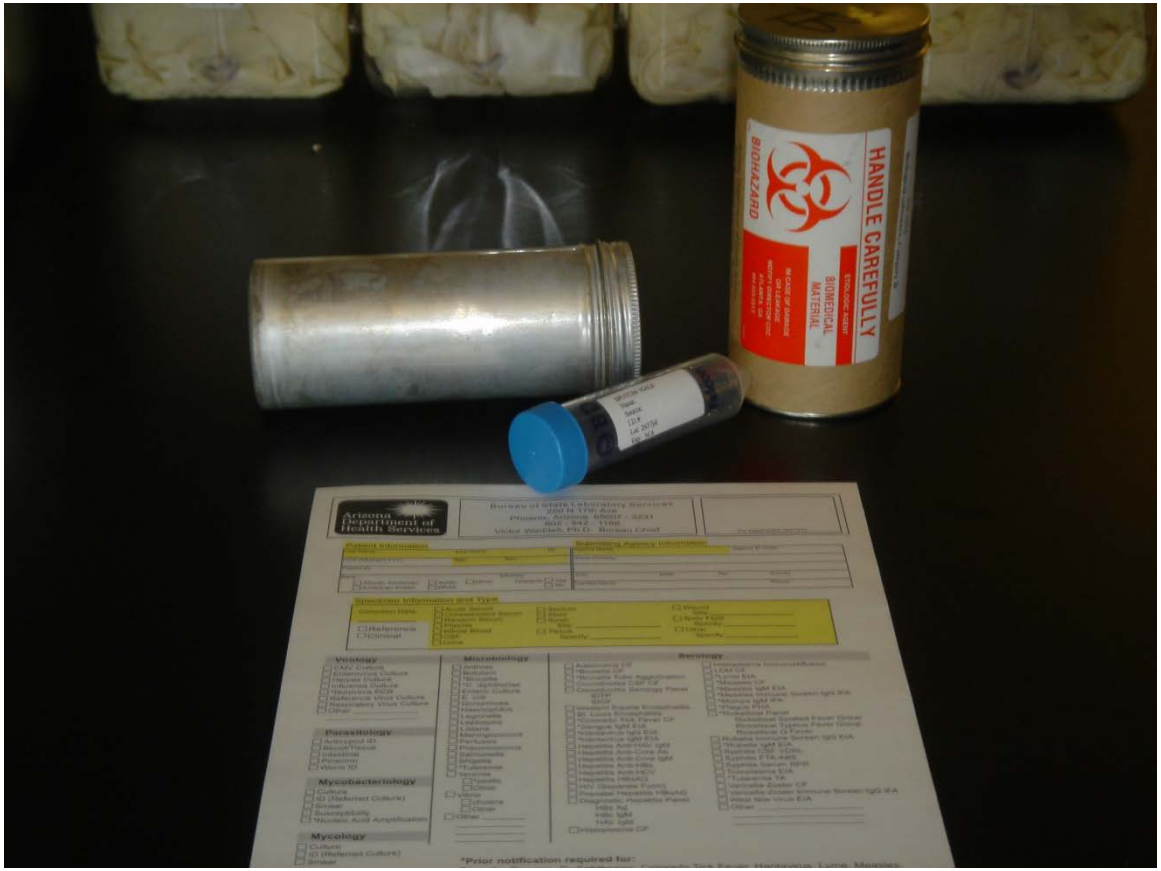
- Diagnostic and Reference laboratory
- Hours of Operation: Monday – Friday, 8:00am -5:00pm
- AZDHS Website http://www.azdhs.gov/lab/micro/index.htm
  - Guide to Laboratory Services
  - Laboratory Submission form

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
  - http://www.azdhs.gov/lab/micro/pdf/micro-submission-form.pdf

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

Last name: First name: MI:
DOB (MM/DD/YYYY): Age: Sex: ☐M ☐F ☐T Patient ID:
Street address: City: State: Zip: County: select one
Ethnicity: Hispanic ☐ Yes ☐ No Race: ☐White ☐African American ☐Asian ☐American Indian/Alaska Native ☐Other

SUBMITTING AGENCY INFORMATION

Agency name: Agency ID Code:
Street address: City: State: Zip: County: select one
Contact name: Tel:

ORDERING PROVIDER INFORMATION

Provider name: Tel:
Facility name: Tel:
Street address: City: State: Zip: County: select one

SPECIMEN INFORMATION & TYPE

Collection date:

☐Serum ☐Acute ☐Convalescent	☐Plasma	☐Whole blood	☐CSF
☐Swab, site:	☐Urine	☐Sputum	☐Stool
Other, specify:	☐Tissue, specify:	☐Wound, site:	☐Body fluid, specify:

☐Clinical ☐Reference: ☐Broth ☐Isolate Reason for testing: ☐Outbreak ☐Surveillance ☐Post Mortem ☐Screening ☐Diagnostics
Outbreak name:

Laboratory Submission Form

Contact Name: _____

VIROLOGY

- ☐ CMV Culture
- ☐ Enterovirus Culture
- ☐ Herpes Culture
- ☐ Influenza
- ☐ Norovirus PCR
- ☐ Reference Virus Culture
- ☐ Respiratory Virus Culture
- ☐ Other _____

SELECT AGENTS**

- ☐ Avian Influenza H5N1
- ☐ *Bacillus anthracis
- ☐ *Brucella spp.
- ☐ *Burkholderia spp.
- ☐ *Francisella tularensis
- ☐ *Orthopox
- ☐ *Q Fever
- ☐ *SARS
- ☐ *Yersinia pestis

**Please refer to the Guide to Laboratory Services: Microbiology, Section 8 for the definition of select agents and the testing available at Arizona State Laboratory

BACTERIOLOGY

- ☐ *Bordetella pertussis
- ☐ Campylobacter spp.
- ☐ *Clostridium botulinum toxin
- ☐ *Corynebacterium diphtheriae
- ☐ Enteric culture
- ☐ Escherichia coli / Shigatoxin
- ☐ Haemophilus influenzae
- ☐ Legionella spp.
- ☐ Leptospira spp.
- ☐ Listeria spp.
- ☐ Neisseria gonorrhoeae
- ☐ Neisseria meningitidis
- ☐ Salmonella spp.
- ☐ Shigella spp.
- ☐ Vibrio
- ☐ VISA/VRSA
- ☐ Yersinia (Non-pestis) Culture
- ☐ Other: _____

PARASITOLOGY†

- ☐ Arthropod ID
- ☐ Blood/Tissue
- ☐ Intestinal
- ☐ Pinworm
- ☐ Worm ID

† For malaria testing please collect patient travel history

MYCOBACTERIOLOGY

- ☐ Culture
- ☐ ID (Referred Culture)
- ☐ *Nucleic Acid Amplification
- ☐ Smear
- ☐ Susceptibility

SEROLOGY

- ☐ *Borrelia burgdorferi EIA (Lyme)
- ☐ *Brucella Tube Ag.
- ☐ Coccidioides Serology Panel ☐ IDTP ☐ IDCF
- ☐ Dengue IgG EIA
- ☐ Diagnostic Hepatitis Panel EIA ☐ HBsAG ☐ HBcIgM ☐ HAV IgM
- ☐ *Francisella tularensis Tube Ag.
- ☐ *Hantavirus IgG EIA
- ☐ *Hantavirus IgM EIA
- ☐ Hepatitis Anti-HAV IgM
- ☐ Hepatitis Anti-Core IgM
- ☐ Hepatitis Anti-HCV
- ☐ Hepatitis HBsAG
- ☐ *Measles IgM EIA
- ☐ Mumps IgM EIA
- ☐ Rickettsial Panel IFA ☐ Rickettsial Q Fever ☐ Rickettsial Spotted Fever ☐ Rickettsial Typhus Fever
- ☐ *Rubella IgM EIA
- ☐ St. Louis Encephalitis EIA
- ☐ Treponema pallidum CSF VDRL
- ☐ Treponema pallidum Serum TP-PA, RPR
- ☐ Western Equine Encephalitis EIA
- ☐ West Nile Virus EIA
- ☐ *Yersinia pestis PHA
- ☐ Other: _____

Submitting 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

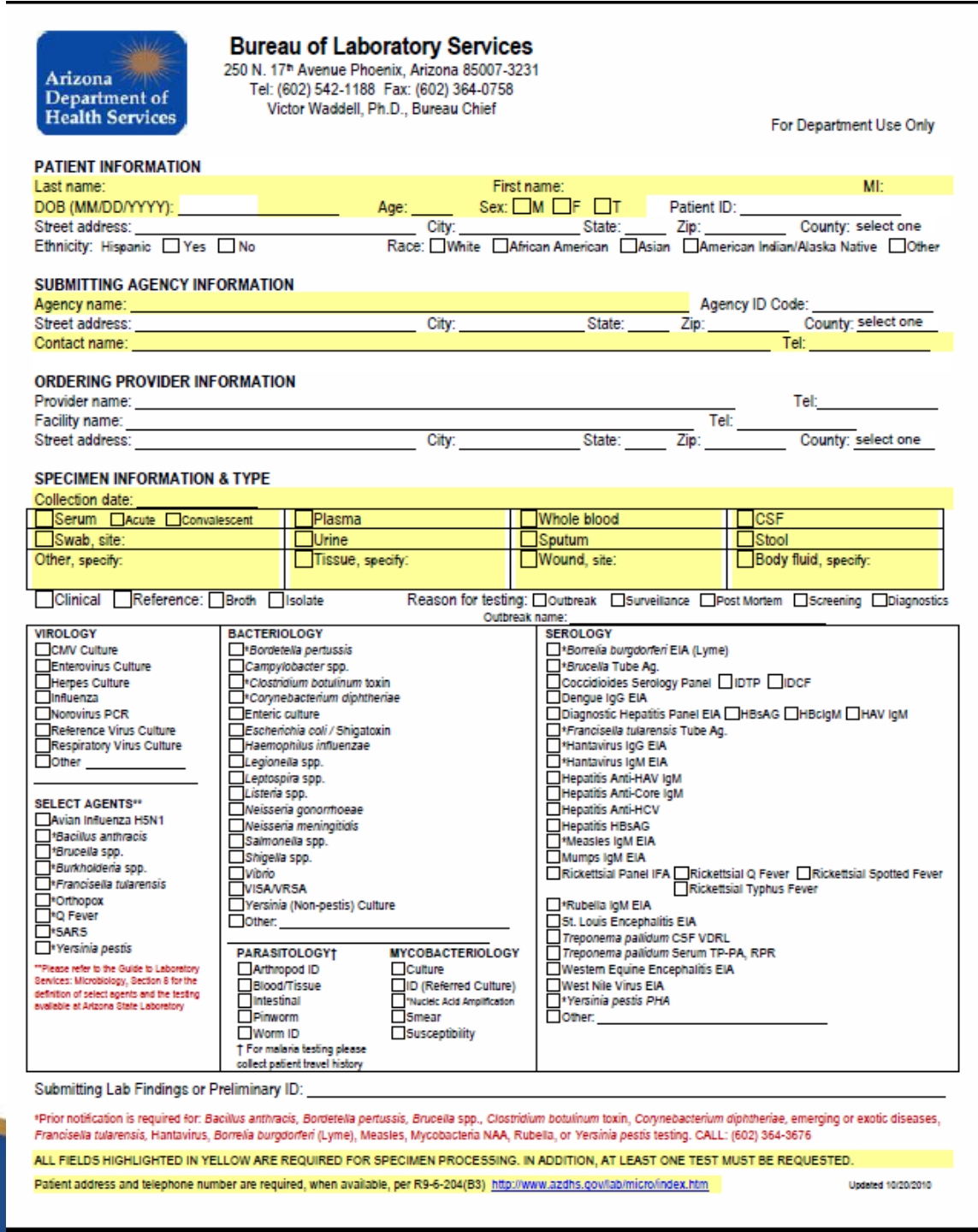
ALL FIELDS HIGHLIGHTED IN YELLOW ARE REQUIRED FOR SPECIMEN PROCESSING. IN ADDITION, AT LEAST ONE TEST MUST BE REQUESTED.

Patient address and telephone number are required, when available, per R9-6-204(B3) http://www.azdhs.gov/lab/micro/index.htm

Updated 10/20/2010

Laboratory Submission Form

- Complete all highlighted (required) fields



Arizona Department of Health Services

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
Last name: First name: MI:
DOB (MM/DD/YYYY): Age: Sex: ☐M ☐F ☐T Patient ID:
Street address: City: State: Zip: County: select one
Ethnicity: Hispanic ☐ Yes ☐ No Race: ☐White ☐African American ☐Asian ☐American Indian/Alaska Native ☐Other

SUBMITTING AGENCY INFORMATION
Agency name: Agency ID Code:
Street address: City: State: Zip: County: select one
Contact name: Tel:

ORDERING PROVIDER INFORMATION
Provider name: Tel:
Facility name: Tel:
Street address: City: State: Zip: County: select one

SPECIMEN INFORMATION & TYPE
Collection date:

☐Serum ☐Acute ☐Convalescent	☐Plasma	☐Whole blood	☐CSF
☐Swab, site:	☐Urine	☐Sputum	☐Stool
Other, specify:	☐Tissue, specify:	☐Wound, site:	☐Body fluid, specify:

☐Clinical ☐Reference: ☐Broth ☐Isolate Reason for testing: ☐Outbreak ☐Surveillance ☐Post Mortem ☐Screening ☐Diagnostics
Outbreak name:

VIROLOGY
☐CMV Culture
☐Enterovirus Culture
☐Herpes Culture
☐Influenza
☐Norovirus PCR
☐Reference Virus Culture
☐Respiratory Virus Culture
☐Other

SELECT AGENTS**
☐Avian Influenza H5N1
☐*Bacillus anthracis*
☐*Brucella* spp.
☐*Burkholderia* spp.
☐*Francisella tularensis*
☐Orthopox
☐*Q Fever
☐*SARS
☐*Yersinia pestis*
**Please refer to the Guide to Laboratory Services: Microbiology, Section B for the definition of select agents and the testing available at Arizona State Laboratory

BACTERIOLOGY
☐**Bordetella pertussis*
☐*Campylobacter* spp.
☐**Clostridium botulinum* toxin
☐**Corynebacterium diphtheriae*
☐Enteric culture
☐*Escherichia coli* / Shigatoxin
☐*Haemophilus influenzae*
☐*Legionella* spp.
☐*Leptospira* spp.
☐*Listeria* spp.
☐*Neisseria gonorrhoeae*
☐*Neisseria meningitidis*
☐*Salmonella* spp.
☐*Shigella* spp.
☐*Vibrio*
☐VISA/VRSA
☐*Yersinia* (Non-pestis) Culture
☐Other:

PARASITOLOGY†
☐Arthropod ID
☐Blood/Tissue
☐Intestinal
☐Pinworm
☐Worm ID
† For malaria testing please collect patient travel history

MYCOBACTERIOLOGY
☐Culture
☐ID (Referred Culture)
☐*Nucleic Acid Amplification
☐Smear
☐Susceptibility

SEROLOGY
☐**Borrelia burgdorferi* EIA (Lyme)
☐**Brucella* Tube Ag.
☐Coccidioides Serology Panel ☐IDTP ☐IDCF
☐Dengue IgG EIA
☐Diagnostic Hepatitis Panel EIA ☐HBsAG ☐HBcIgM ☐HAV IgM
☐**Francisella tularensis* Tube Ag.
☐*Hantavirus IgG EIA
☐*Hantavirus IgM EIA
☐Hepatitis Anti-HAV IgM
☐Hepatitis Anti-Core IgM
☐Hepatitis Anti-HCV
☐Hepatitis HBsAG
☐*Measles IgM EIA
☐Mumps IgM EIA
☐Rickettsial Panel IFA ☐Rickettsial Q Fever ☐Rickettsial Spotted Fever ☐Rickettsial Typhus Fever
☐*Rubella IgM EIA
☐St. Louis Encephalitis EIA
☐*Treponema pallidum* CSF VDRL
☐*Treponema pallidum* Serum TP-PA, RPR
☐Western Equine Encephalitis EIA
☐West Nile Virus EIA
☐**Yersinia pestis* PHA
☐Other:

Submitting 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.

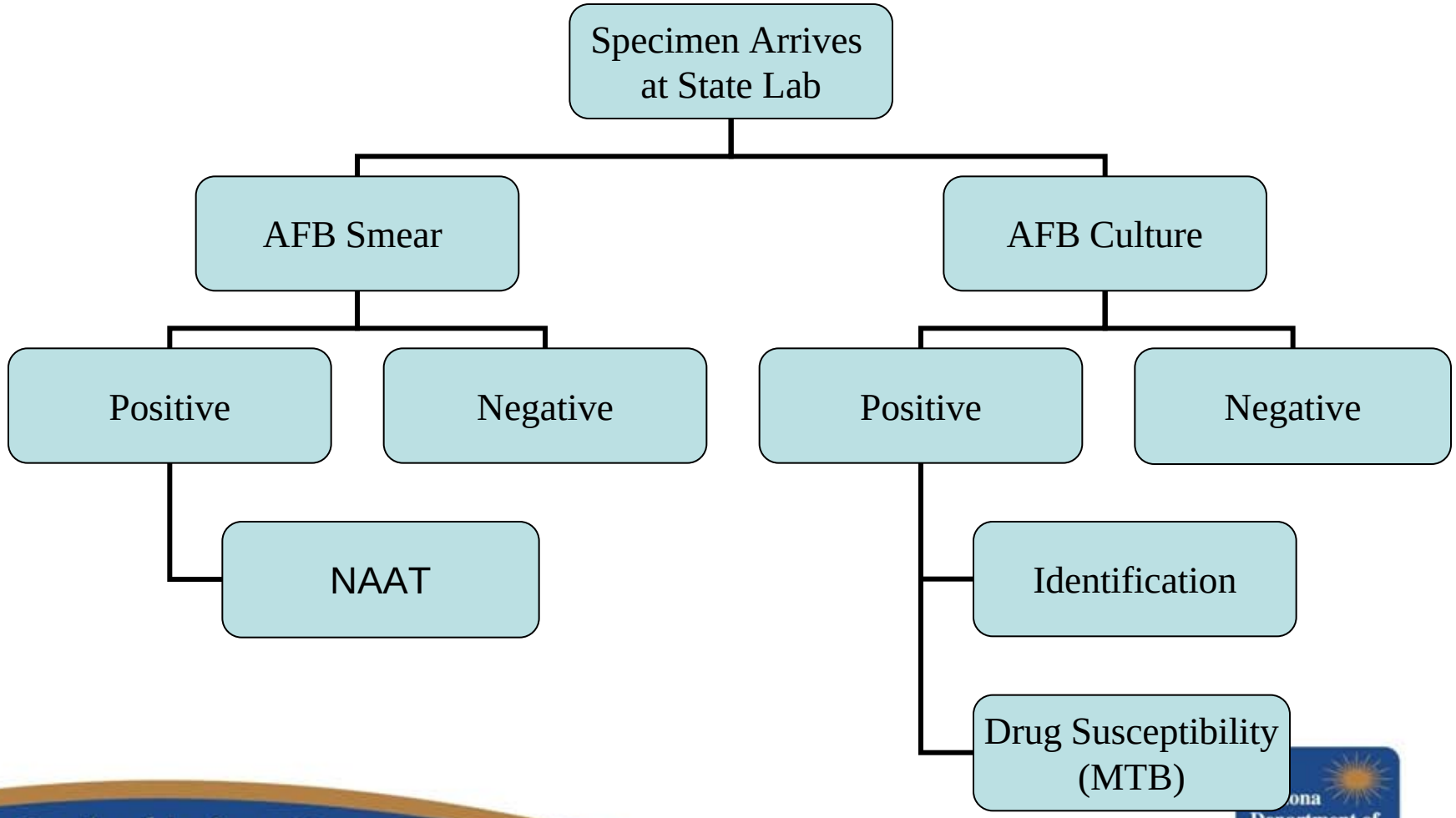
Patient address and telephone number are required, when available, per R9-6-204(B3) http://www.azdhs.gov/lab/microfindex.htm

Updated 10/20/2010

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
  - http://www.azdhs.gov/lab/micro/index.htm
- CDC recommendation:
  - Specimen received at laboratory within 24 hours of specimen collection.

Typical Specimen Workflow at ASL



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

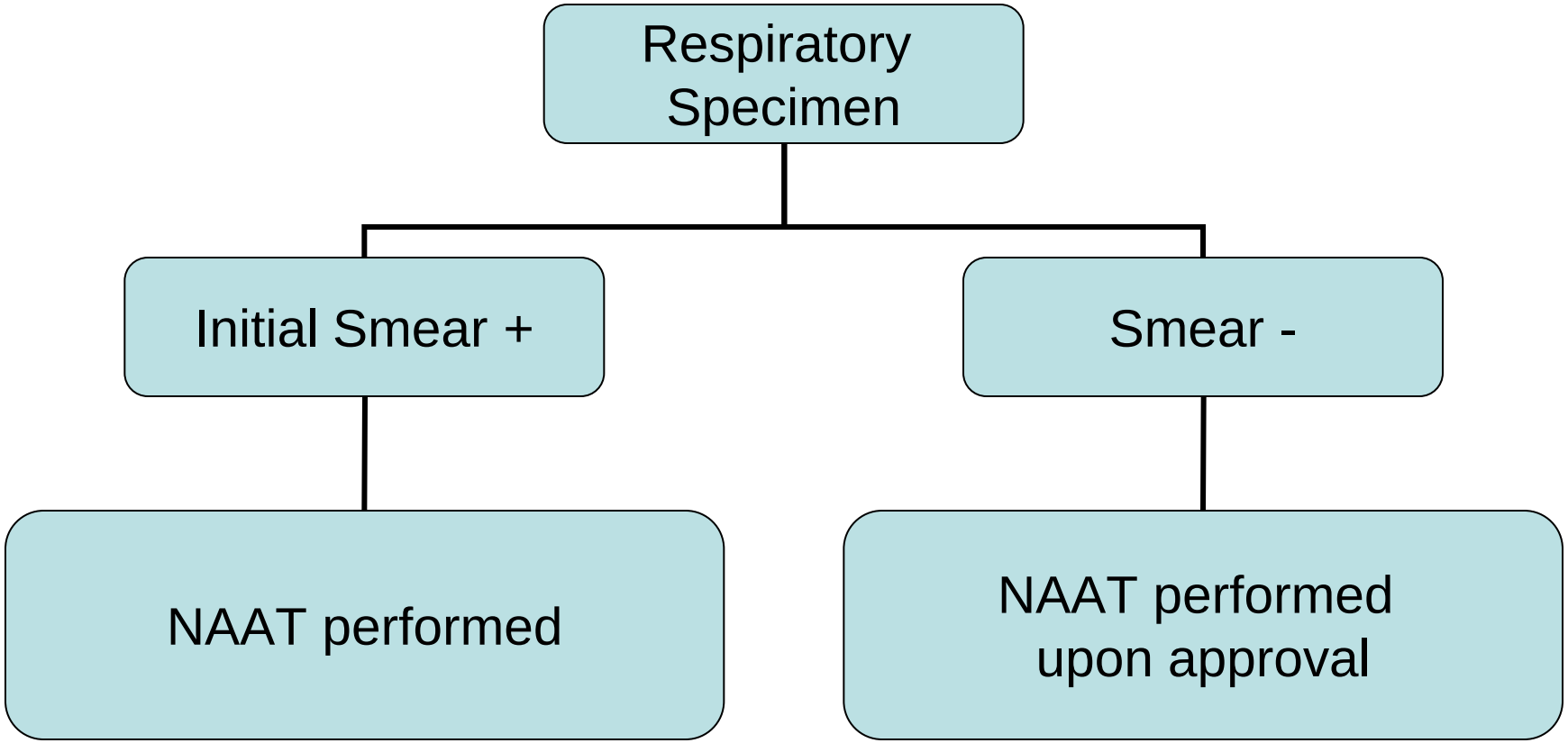
Nucleic Acid Amplification Tests

- 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



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

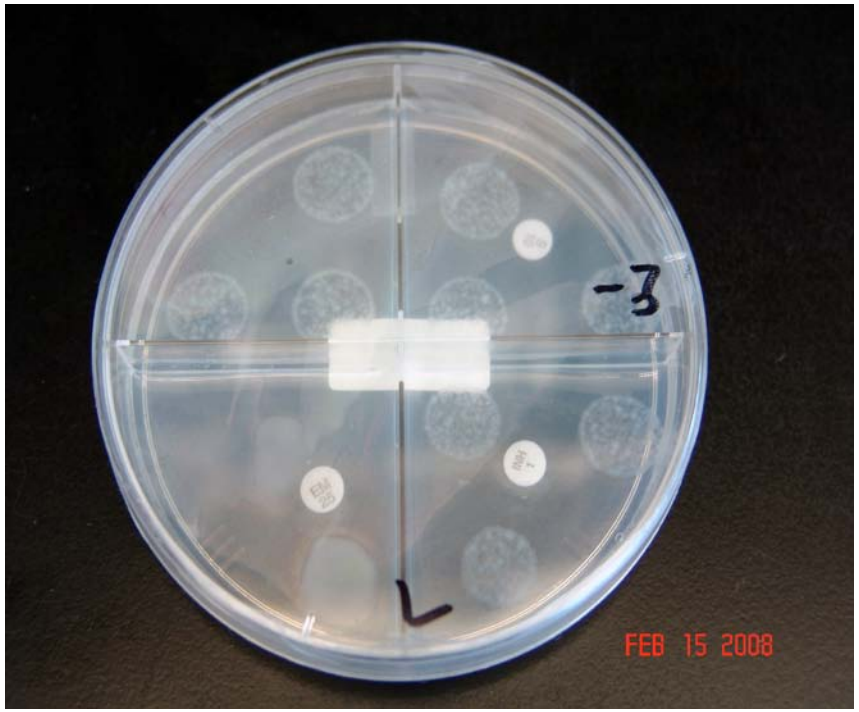
TB Drug Susceptibility Testing

- 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

- The CDC began offering Molecular Detection of Drug Resistance for *Mycobacterium tuberculosis, Sept 2009.*
- 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)

Drug	Gene
RIF	*rpoB*
INH	*inhA, katG*
FQ	*gyrA*
KAN	*rrs, eis*
AMK	*rrs*
CAP	*rrs, tlya*
EMB	*embB*
PZA	*pncA*

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???