

Bioterrorism: Laboratory Zoonotic Disease Surveillance

Detection and Speciation of Select Agent

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Leadership for a Healthy Arizona

State Laboratory

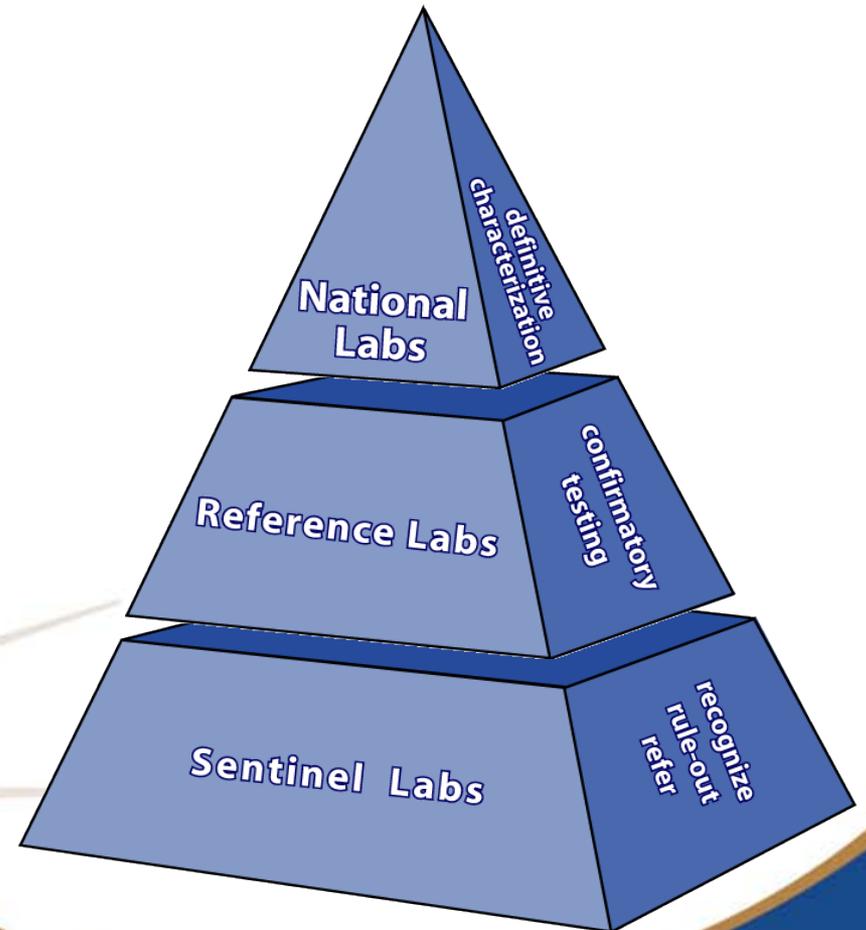
- Located west of Downtown Phoenix
- Wide variety of testing
 - Chemical
 - Biological
 - Virology, Serology, Bacteriology, Environmental (Food and water)
 - Bioemergency
 - Select agents, outbreak assistance (H1N1), assay development



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How are the Laboratories Involved?

- Sentinel Labs
 - Hospital labs, veterinary labs, food labs, environmental labs, county public health labs
- Reference Labs
 - State of Arizona Public Health Lab, Phoenix
- National Labs
 - CDC, USAMRIID



LRN Structure for Bioterrorism

Clinical Testing Capabilities

- *Bacillus anthracis*
- *Brucella melitensis*
- *Brucella suis*
- *Brucella abortus*
- *Brucella canis*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- *Rickettsia rickettsii*
- *Francisella tularensis*
- *Yersinia pestis*
- *Coxiella burnetii*
- Orthopox viruses
- *Clostridium botulinum* Toxin
- Ricin Toxin
- Staphylococcal Endotoxin B
- Avian Influenza (H5)

Sample Types

- Testing performed by Bioemergency Response section
 - Clinical specimen
 - Associated/unassociated with a bioterrorism event
 - Cleared by state and county Epidemiology
 - Reference isolate select agents
 - Call ahead notification
 - Environmental unknowns
 - Water, soil, vectors (mosquitoes, flies, fleas and ticks)
 - Threat letters



Organism / Disease	Specimen Type
B. anthracis (Anthrax)	PCR: Blood, serum, Isolate (liquid or plated), plasma, pleural fluid, transtracheal aspirate, sputum, tissue Organism isolation: vesicular fluid, swab of eschar material, blood, sputum, stool, lymph node biopsy
Brucella spp.* (Brucellosis)	PCR: Whole Blood (200 µl min) Isolate (liquid or plated) Organism isolation: Blood, bone marrow, spleen, liver, abscess
Burkholderia spp. (Glanders– <i>B. mallei</i>) (Melioidosis– <i>B. pseudomallei</i>)	PCR: Whole Blood (200 µl min) Serum (200 µl min) Isolate (liquid or plated) Organism isolation: Blood, urine, abscess, tissue aspirates, isolates
C. Burnetii* (QFever)	PCR: Whole Blood (200 µl min)

Acceptable Specimens:
 Table taken from Arizona Dept. Health Services
 "Guide to Laboratory Services"
<http://azdhs.gov/lab/micro/labguide.pdf>

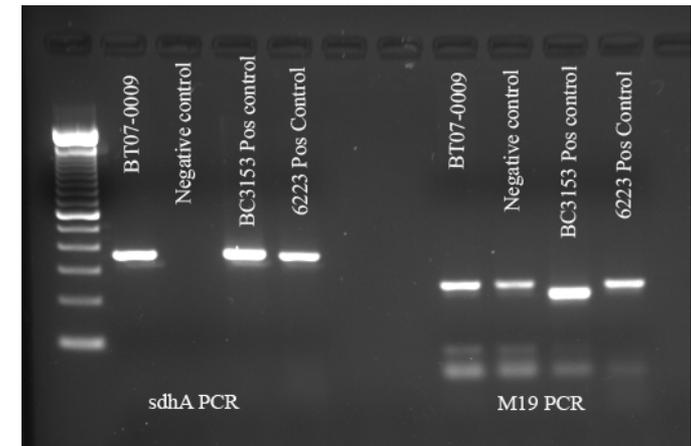
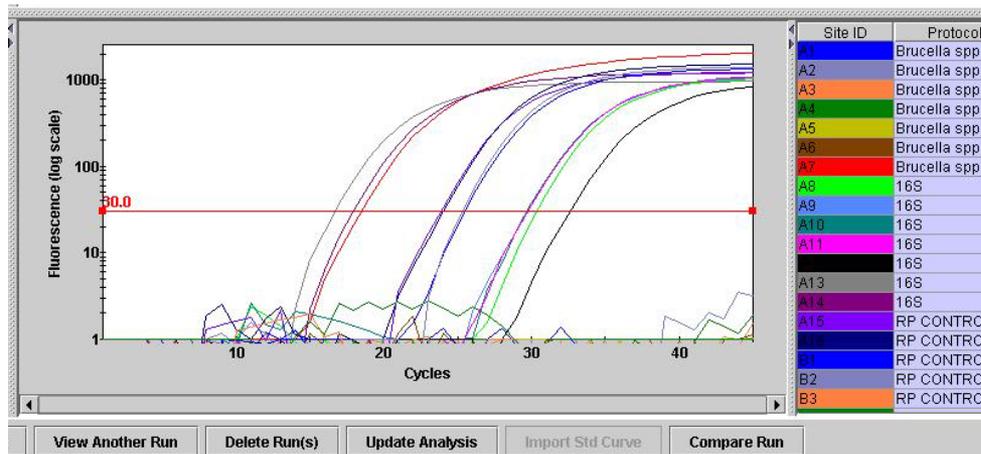
Organism / Disease	Specimen Type	
F. tularensis* (Tularemia)	<p>PCR: Whole Blood (200 µl min) Isolate (liquid or plated)</p> <p>DFA: isolate (liquid or plated), ulcer swab, aspirate, tissues, bronchial/tracheal wash, pleural fluid, sputum, abscess material, bone marrow scrapings</p> <p>Organism isolation: blood, tissue biopsy, aspirates, ulcer scrap/swab</p>	<p>Acceptable Specimens: Table taken from Arizona Dept. Health Services "Guide to Laboratory Services" http://azdhs.gov/lab/micro/labguide.pdf</p>
Influenza A/H5	<p>PCR: nasopharygeal swabs and/or aspirates, oropharyngeal aspirates and/or washes, throat swabs, sputum, tracheal aspirate, BAL, viral culture</p>	
Orthopox	<p>PCR: Dried vesicle fluid on a slide, fresh biopsy, skin or crust from roof of vesicle, swab of lesion (dry or wet)</p>	
Y. pestis* (Plague)	<p>PCR: Isolate (liquid or plated), bronchial wash, transtracheal aspirate, sputum, nasopharyngeal swab</p> <p>DFA: lymphoid aspirate, tissue smear, tissue biopsy, blood in blood culture bottle, bronchial/tracheal wash, isolate, bone</p> <p>Organism isolation: Bronchial wash, transtracheal aspirate, sputum, blood, tissue</p>	

Types of testing

- Molecular

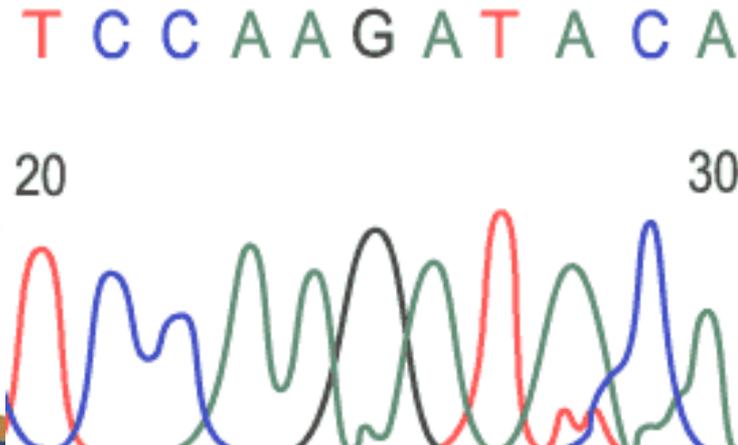
- PCR (Polymerase Chain Reaction)

- Detection
 - Speciation - *F. tularensis*, *Rickettsia* (*typhus* group vs. *spotted fever* group)



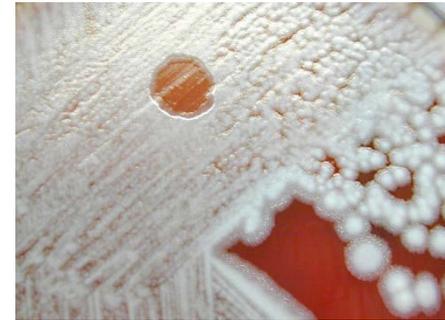
- Sequencing

- *Rickettsia* group speciation
 - *F. tularensis*
 - Miscellaneous bacteria

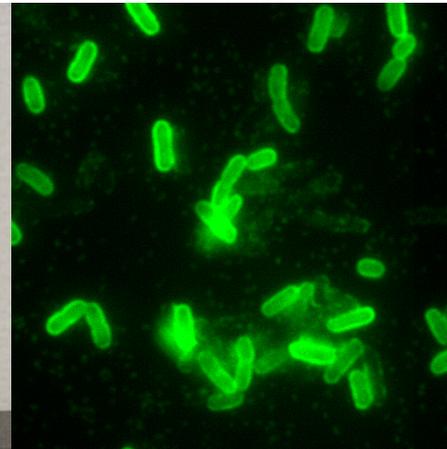
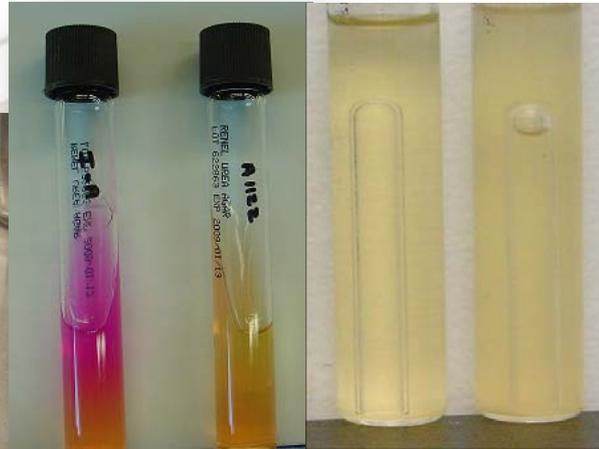


Types of testing

- Conventional
 - Traditional bacterial culture
 - Direct Fluorescent Antibody (DFA) Stain
 - Biochemical
 - Phage testing
 - Dye tolerance
 - Capsule production
 - Gas production
 - Consult algorithm/reference tables

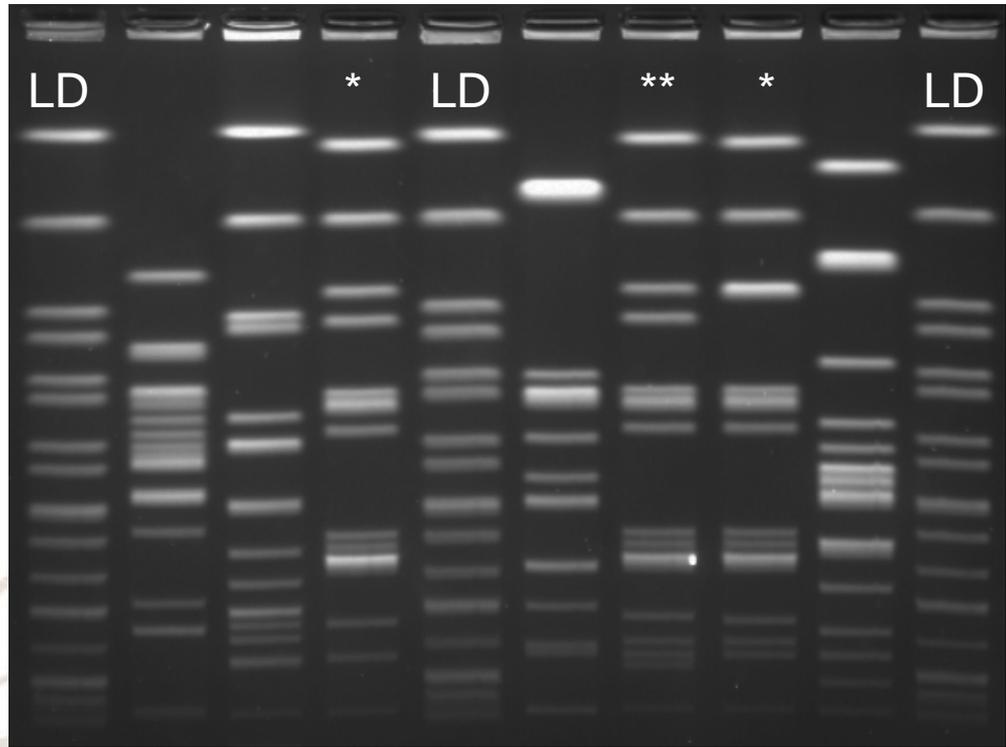


CDC PHIL / Courtesy of Larry Stauffer, Oregon State Public Health Laboratory



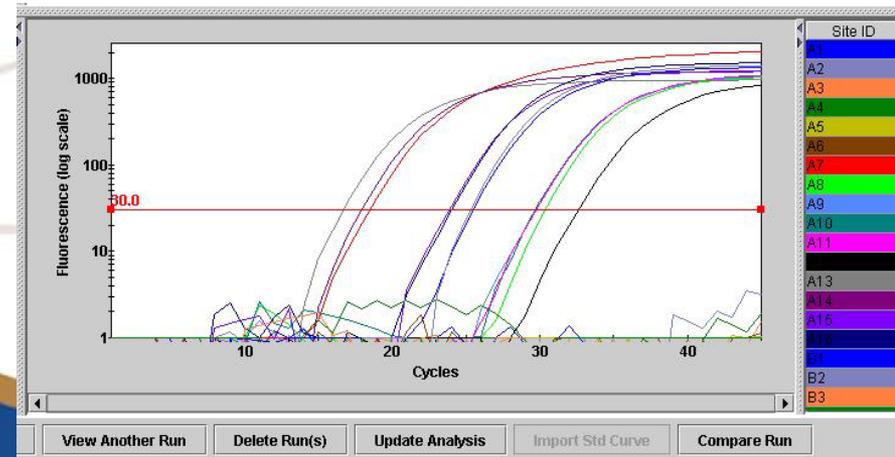
Pulse Field Gel Electrophoresis (PFGE)

- Used to associate organism/strain with outbreak
- Isolate DNA digested and compared
- *Yersinia pestis*,
E. coli, *Salmonella*



Additional testing

- Special request
 - Facilitate epidemiological investigation
 - Rickettsia spp.
 - Typhus Group
 - » Clinical material from pack rats
 - Spotted Fever Group
 - » Ticks
 - » 40-50 samples submitted, 1 positive



Reporting

- Results
 - Reported as presumptive or confirmed
 - Based on test performed
 - PCR only vs. biochemical test
 - Select agents reported
 - Original submitter
 - State and County Epidemiology
 - CDC

Recent HAN

(June 10, 2010)

- Brucellosis Update
- This HAN is being issued to physicians and laboratory workers to alert them of the apparent increase in *Brucella* infections being reported in Arizona. To date, four cases of brucellosis have been reported in Arizona in 2010, including two each in Maricopa and Pima counties. Four cases is above normal this early in the year. Also, possible *Brucella* exposures to laboratory staff have occurred in three Arizona hospitals where staff worked with *Brucella* cultures outside of BSL-3 conditions. There is a similar increase being reported south of the border. Additional brucellosis cases are likely to be diagnosed in the weeks and months ahead. When submitting specimens to the laboratory for culture, physicians need to clearly indicate 'SUSPECT OR RULE-OUT BRUCELLA' so that laboratory staff will know to follow appropriate protocols to avoid laboratory exposure.
- Brucellosis should be considered in the differential diagnosis of compatible illness in patients who have recently traveled out-of-country (especially Mexico and other Latin American countries), or consumed unpasteurized dairy foods such as queso fresco cheese (goat cheese) or raw milk. The unpasteurized foods are often consumed during travel south of the border or are homemade food products (e.g. goat cheese) that are brought into Arizona by visiting friends or relatives. Suspected cases of brucellosis should be reported immediately to the local health agency.
- Diagnosis is made through culture from clinical specimens (blood, abscess, bone marrow, tissue) or through serologic testing showing a rise in *Brucella* specific antibodies. Positive laboratory results (especially *Brucella* cultures) should be reported same day to the Arizona Department of Health Services (ADHS), and specimens/isolates should be sent to the Arizona State Health Laboratory (ASHL) for PCR and culture. Serologic testing is also an option but does not allow for *Brucella* speciation. Agglutination testing (e.g. tube agglutination or microagglutination) is the gold standard for serology and is offered at the ASHL. Some of the commercially available serologic tests (especially IgM EIA) have been known to produce false positive results. For this reason, follow-up testing at the ASHL is often necessary to confirm or rule-out brucellosis. For more information see the attached Physician Fact Sheet, CDC Recommendations for Brucella Laboratory Exposures and CDC Risk Assessment Table.



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Resources

- Contacts
 - State Epidemiology (Vector/Zoonotic)
 - Link to “Guide to Laboratory Services”
 - <http://azdhs.gov/lab/micro/labguide.pdf>
 - State Laboratory Bioemergency Section:
 - (602)364-0999
 - Rule-out and Refer Class or Packaging and Shipping of infectious materials
 - Contact Rob Nickla
 - (602)364-0530

