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**Guide to Laboratory Services: Microbiology**

**Arizona Department of Health Services  
Bureau of State Laboratory Services  
250 North 17<sup>th</sup> Avenue  
Phoenix, Arizona 85007  
(602) 542-1188**

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Victor Waddell Ph.D.  
Bureau Chief  
Daniel M. Lavine, M.D.  
Laboratory Director  
William M. Slanta  
Assistant Bureau Chief

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## General Information

Bureau Chief, Laboratory Services

Victor Waddell Ph.D.

Director, Laboratory Services

Daniel M. Lavine, M.D.

Assistant Bureau Chief

William M. Slanta

Hours of Operation: 8:00 AM to 5:00 PM Monday through Friday (Emergency services available on nights or weekends when required by public health needs.)

Receiving section only is open from 9:30am to 4:30pm on Saturday

Annual Holiday Schedule: Laboratory Services observes all state recognized holidays.

Location: 250 North 17<sup>th</sup> Avenue, Phoenix, Arizona 85007

Telephone Number: (602) 542-1188

WATTS Line: (800) 525-8915

Fax Number: (602) 542-0760

Emergency Phone  
(Weekends/After Hours): (480) 303-1676

## Arizona State Public Health Laboratory Contact Information

<b>Section</b>	<b>Supervisor</b>	<b>Telephone Number</b>
Receiving /Shipping	Kathleen Rodriguez	(602) 542-1190
TB /Mycobacteriology/ Molecular Methods Research	Stacy White, Ph.D.	(602) 542-6131
Virology/ Serology	Kathryn Fitzpatrick	(602) 542-0968
Bioemergency Response and Detection for Select Agents	J. Gage Patterson, M.S.	(602) 364-0999
Bacteriology / (Limited) Parasitology		(602) 542-6132
Environmental Microbiology	Roumen Penev, Ph.D.	602) 542-6130

## Core Functions and Capabilities of State Public Health Laboratories

State public health laboratories face the broad challenge of working towards prevention and control of disease and improvement of health. To function in this capacity, the public health labs provide testing for, and aid in the diagnosis of, unusual pathogens. The labs serve as the first line of defense in the rapid recognition and prevention of the spread of communicable diseases, while also serving as centers of expertise for the detection and identification of biologic agents of importance in human disease. The public health labs also perform testing to meet the specific program needs of the public health agencies.

### **Routine diagnostic testing for hospitals and private laboratories is provided through independent reference laboratories.**

The policy of the Arizona State Public Health Laboratory (ASPHL) is to provide microbiology and immunology diagnostic support to county and state agencies. In addition, the ASPHL serves as a reference microbiology laboratory to hospital and independent clinical laboratories in order to confirm their atypical results from cultures and clinical specimens. This information is also used as part of the Department of Health Services disease surveillance program. Selected diagnostic test procedures are available to private medical practitioners when a procedure is not available through independent reference laboratories or when intense surveillance is deemed necessary. The laboratory also accepts food and water from county and state agencies for outbreak investigations and surveillance.

ASPHL reporting requirements can be found at <http://www.azdhs.gov/phs/oids/reporting/labs.htm> . This report identifies agents which must be reported to the state and which isolates must be submitted to the laboratory. Please follow packing guidelines found in this manual, or on our website at <http://www.azdhs.gov/lab/>

The ASPHL provides specimen collection materials and mailers free of charge. Further information regarding specimen collection materials, mailing containers and *Request for Materials Form* is located in **Section 09: Requesting Collection Kits and Mailing Containers**. All requisitions and supplies for specimen submission are available through the Receiving Section in Phoenix at <http://www.azdhs.gov/lab/shipping-receiving.htm>.

The purpose of this manual is to provide a ready reference to our clients and to assist them in obtaining laboratory services as efficiently as possible. Charts are provided for quick reference and more detailed information is available by test or organism name in each section of the manual. This manual can be downloaded or viewed at <http://www.azdhs.gov/lab/documents/microbiology/lab-guide.pdf>

## Specimen Rejection Policy

The ASPHL currently has the following policy for rejection of laboratory specimens and/or requested examinations. The ASPHL will NOT examine clinical/reference specimens if the following circumstances exist:

- Test is routinely available at a hospital or a private independent laboratory
- The identifier on the specimen did not match the identifier on the submission form, or there was no identification on the specimen.
- The quantity of specimen was not sufficient for examination.
- The specimen was too long in transit between the time of collection and receipt in the laboratory.
- The specimen was broken or leaked in transit.
- Clinical/epidemiological information submitted with the specimen was either insufficient or incomplete.
- Specimen was submitted in an improper or expired container, transport media or preservative.
- Blood specimens were hemolyzed or contaminated.
- Only acute blood specimen was submitted, no convalescent specimen.
- Material for rabies examination was too decomposed or desiccated to test.
- Reference cultures were mixed or contaminated; only pure cultures are acceptable.
- Tissues were not submitted in individual containers
- Test request deemed unnecessary by the Bureau of Epidemiology

Exceptions to this policy will be considered due to extenuating circumstances; however, final approval to make an exception can only be made by the Laboratory Director, Bureau Chief, Assistant Bureau Chief, or Technical Supervisor.

## Directory of Laboratory Services

The following table lists the diagnostic and reference services offered by the Office of Public Health Microbiology. The table is organized alphabetically by disease or agent for easy referral. Please go to the specified laboratory section of this manual for more detailed information on collection and submission of laboratory samples.

Disease or Agent	Culture	Antigen Detection	Toxin	Smear	Serology Single Serum	Serology Paired Sera	Laboratory Section	Manual Location
Adenovirus	X						Virology	Section 5
Anthrax <i>Bacillus anthracis</i>	X	PCR					Bioemergency	Section 8
<i>Bacillus cereus</i>	X						Environmental	Section 7
Blood/Tissue Parasites				X			Parasitology Referred to CDC	Section 3
Botulism <i>Clostridium botulinum</i>	X		X				Bacteriology Referred to CDC	Section 1
<i>Brucella</i>	X	PCR					Bioemergency	Section 8
<i>Burkholderia mallei/</i> <i>pseudomallei</i>	X	PCR					Bioemergency	Section 8
* <i>Campylobacter</i>	X						Bacteriology	Section 1
Chikungunya		PCR			X		Serology	Section 4
Cytomegalovirus (CMV)	X						Virology	Section 5
Dengue					X		Serology	Section 4
Diphtheria <i>Corynebacterium diphtheriae</i>	X						Bacteriology	Section 1
<i>E. coli</i>	X						Bacteriology Environmental	Section 1 Section 7
Enterovirus	X						Virology	Section 5
Hantavirus					X	X	Serology	Section 4
<i>Haemophilus</i>	X						Bacteriology	Section 1
Hepatitis C					X		Serology	Section 4
HIV					X		Serology	Section 4
Influenza	X	PCR					Virology	Section 5

Disease or Agent	Culture	Antigen Detection	Toxin	Smear	Serology Single Serum	Serology Paired Sera	Laboratory Section	Manual Location
Intestinal Parasites (Giardia and Cryptosporidium)				X			Parasitology	Section 3
<i>Legionella</i>	X						Bacteriology Referred to CDC	Section 1
<i>Leptospira</i>	X						Bacteriology Referred to CDC	Section 1
<i>Listeria</i>	X						Bacteriology Environmental	Section 1 Section 7
Lyme Disease <i>Borrelia</i>					X		Serology Referred to CDC	Section 4
Measles	X	PCR				X	Virology Serology	Section 5 Section 4
<i>Neisseria meningitidis</i>	X						Bacteriology	Section 1
Mumps	X	PCR				X	Virology Serology	Section 5 Section 4
Murine Typhus <i>Rickettsia</i>						X	Serology	Section 4
<i>Mycobacterium</i>	X	HPLC NAAT		X			Mycobacteriology	Section 2
Norovirus		PCR					Virology	Section 5
Orthopoxvirus		PCR					Bioemergency	Section 8
Parainfluenza viruses (1-4)	X						Virology	Section 5
Pertussis <i>Bordetella pertussis</i>	X						Bacteriology	Section 1
Q Fever <i>Coxiella burnetii</i>		PCR				X	Bioemergency Serology	Section 8 Section 4
Rabies				X			Virology	Section 5
Rocky Mountain Spotted Fever <i>Rickettsia</i>						X	Serology	Section 4

Disease or Agent	Culture	Antigen Detection	Toxin	Smear	Serology Single Serum	Serology Paired Sera	Laboratory Section	Manual Location
Respiratory Syncytial Virus (RSV)	X						Virology	Section 5
Rubella					X		Serology	Section 4
<i>Salmonella</i>	X						Bacteriology Environmental	Section 1 Section 7
Shiga toxin	X		PCR				Bacteriology	Section 1
* <i>Shigella</i> (For identification and confirmation only)	X						Bacteriology Environmental	Section 1 Section 7
St. Louis Encephalitis		PCR**			X		Serology	Section 4
<i>Staphylococcus</i> (VISA/VRSA)	X						Bacteriology	Section 1
Syphilis					X		Serology	Section 4
Tularemia <i>Francisella tularensis</i>	X	PCR					Bioemergency	Section 8
Varicella-Zoster	X						Virology	Section 5
<i>Vibrio</i>	X						Bacteriology Environmental	Section 1 Section 7
West Nile		PCR**			X		Serology	Section 4
<i>Yersinia</i>	X	PCR					Bioemergency	Section 8
	X						Bacteriology	Section 1

\*Reference isolates no longer accepted.

HPLC = High Performance Liquid Chromatography

NAAT= Nucleic Acid Amplification

PCR = Polymerase Chain reaction

PCR\*\* on mosquito pools only

\*\*\* On Approval Only

## Section 1: Bacteriology

Upon receipt at the ASPHL, all specimens are logged in and assigned to the appropriate area for processing. The time required to process a microbiology specimen varies considerably, as indicated by the following table. Detailed information on the collection and submission of laboratory samples on any of the following tests can be obtained in the following narrative guidelines.

During outbreaks, the Bureau of Epidemiology and Disease Control may conduct surveillance to determine the extent of the outbreak or to determine the relatedness of microorganisms identified in the outbreak. The Office of Public Health Microbiology will support these outbreak investigations through the use of various molecular tools, and Pulsed Field Gel Electrophoresis (PFGE). Data may be shared in these investigations with other states and the CDC in the event of a multi-state outbreak.

Organism/ Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT) Business Days
Botulism <i>Clostridium botulinum</i>	Serum, Feces, Food	None	Testing requires prior approval by Epidemiology	Referred to CDC
<i>Campylobacter</i>	Feces	Cary-Blair or Modified Cary-Blair	Reference isolates no longer accepted. See Enteric Culture	3-5 days
Diphtheria <i>Corynebacterium diphtheriae</i>	Throat (membrane) or NP Swab	Semi-solid transport media	Call before submitting	4-6 days
Shiga Toxin - producing <i>E. coli</i>	Broth or Pure Culture of Isolate	agar slant or plate; GN or MAC broth	See Enteric Culture	Isolate 4-7 days Broth 6-10 days
Enteric Culture	Feces	Cary-Blair, or Modified Cary-Blair	Includes <i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , and toxin - producing <i>E. coli</i>	5-9 days
<i>Haemophilus</i> ** Serotyping	Pure Culture of Isolate from Sterile Body Site	Chocolate agar slants*	Children 5 years and younger	9-15 days
<i>Legionella</i> **	Pure Culture of Isolate	BCYE plate		Preliminary 3- 4 days Sent to CDC for identification

<b>Organism/ Disease</b>	<b>Specimen</b>	<b>Transport Medium</b>	<b>Comments</b>	<b>Turn Around Time (TAT) Business Days</b>
<i>Leptospira</i>	Blood with Heparin, CSF, Urine	Fletchers Media	Transport at 20° C- 30° C	Preliminary 3-4 days Sent to CDC
<i>Listeria</i> **	Pure Culture of Isolate	Blood agar/or Agar slant	Ship at 4° C	Preliminary 4-8 days Sent to CDC for serotyping
<i>Neisseria meningitidis</i> **	Pure Culture of Isolate from Sterile Body Site	Chocolate agar/or Chocolate Agar slant	Do not refrigerate	3-5 days
Pertussis <i>Bordetella pertussis</i>	Nasopharyngeal Swab or isolate	Reagan-Lowe or Amie's with charcoal	Use polyester NP swabs	PCR Preliminary 2-4 days Culture 8-14 days
<i>Salmonella</i> Serotyping	Pure Culture of Isolate in TSI or Nutrient Agar Slant	Culture Plate/Slant	See Enteric Culture	5-14 days
VISA/VRSA <i>Staphylococcus</i> **	Pure Culture of Isolate	Culture Plate/Slant		5-7 days
<i>Vibrio</i>	Pure Culture of Isolate	Culture Plate/Slant	Do not refrigerate	4-6 days

\*\* Reference cultures only

## **Botulism** *Clostridium botulinum*

### **Collection**

#### **Infant Botulism\***

1. Serum for toxin – 2.5 mL minimum
2. Stool for culture and toxin – 20 to 50 grams (or as much as possible)
  - Toxin testing – 10 to 30 grams
  - Culture – 10 to 20 grams or 15 to 25 mL of watery enema. In some cases, a rectal swab may be accepted, only if other stool specimens are not available.
3. Food for toxin and culture

#### **Food borne Botulism – Adult\***

1. Serum – 15 to 20 mL
2. Feces – 25 to 50 grams
3. Remainder of suspected food

**\* Approval for botulism testing must be obtained from the Bureau of Epidemiology and Disease Control prior to submission. Contact the Infectious Disease Section of the Bureau of Epidemiology and Disease Control at (602) 364-3676 (main number)/480-303-1191 (after hour's number).**

#### **Wound Botulism**

1. Serum – 15 to 20 mL
2. Feces – 25 to 50 grams
3. Tissue, exudate or swab samples from wound

### **Shipment of Specimens**

All specimens should be kept at refrigerated temperatures during storage and shipment. Shipment should contain ice or cool packs.

See Section 8: Sample Submission Guidelines.

All specimens will be forwarded on to the Centers for Disease Control in Atlanta, Georgia for testing.

### **Reporting and Interpretation of Results**

The State Laboratory will notify the submitting agency and the Bureau of Epidemiology and Disease Control with results of the botulism testing as soon as they are available.

## **Diphtheria** *Corynebacterium diphtheriae*

### **Collection**

Both throat swabs and nasopharyngeal swabs should be collected from patients suspected of having Diphtheria. Wound swabs are also acceptable for cutaneous Diphtheria.

The swabs should be placed in semi-solid transport media such as Stuart's media or Amie's gel transport media and sent to the ASPHL to be received within 24 hours of collection.

### **Shipment of Specimens**

The ASPHL must be notified 24 hours in advance (if possible) of a specimen submission. The specimen should be immediately transported to the Arizona State Public Health Laboratory or inoculated onto proper media.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Cultures will be examined for 48 hours and observed daily for typical growth characteristics. Suspicious colonies are checked microscopically for typical morphology, and identification of suspected isolates will be made using biochemical tests. Positive cultures of *C. diphtheriae* are classified into four biotypes based upon their colonial morphology and biochemical reactions. Negative cultures will be held for at least 48 hours before reporting as negative.

Cultures identified positive for *C. diphtheriae* will be sent to CDC for virulence production using in vitro toxigenicity studies.

Positive reports of *C. diphtheriae* will be telephoned to the submitting agency and the Bureau of Epidemiology and Disease Control.

## *E. coli*

See Enteric Culture (Page 1-5), and Shiga Toxin (Page 1-13)

### **Enteric Cultures**

#### **Collection**

The most often cultured sources for enteric diseases are feces, blood, and urine. Other extra-intestinal sources may be infected with enteric disease organisms. Purulent material from wounds or abscesses may be swabbed or aspirated for the presence of *Salmonella sp.*

- Stool specimens should be taken early in the course of illness when the causative agent is likely to be present in the largest numbers. Freshly passed stool is better than rectal swabs since there is less chance of improper collection and mucus and bloodstained portions can be selected for culture. Collect a small portion of feces, approximately the size of a marble, or a swab coated with feces and place in a transport medium. Whenever possible, multiple specimens should be cultured. The ASPHL will provide agencies with Cary-Blair transport medium. Cary-Blair, or modified Cary-Blair, is the best overall transport medium for diarrheal stools. Unpreserved stool is only acceptable if received within two hours of collection.
- Submit reference isolates of *Salmonella* for epidemiological studies. Transfer isolate to a TSI or nutrient agar slant and forward to the ASPHL.

#### **Shipment of Specimens**

Specimens held in transport media should be refrigerated until examined. Transport specimens to the ASPHL at a refrigerated temperature in the proper transport media. Preserved stools should be received and processed within 3 days of collection.

See Section 8: Sample Submission Guidelines.

#### **Reporting and Interpretation of Results**

Stool samples, unless otherwise specified, will be screened for *Salmonella*, *Shigella*, *Campylobacter*, Shiga Toxin - producing *E. coli*, and upon request *Aeromonas*, *Pleisiomonas*, *Yersinia*, and *Vibrio*. Cultures are examined daily for 72 hours for characteristic morphology. Suspect colonies are screened biochemically, and confirmed with serologic agglutination (where applicable). Organisms in the genus *Salmonella* are typed using both somatic and flagellar antisera. All reports of *Salmonella Typhi* are telephoned to the submitting agency and to the Bureau of Epidemiology and Disease Control.

## *Haemophilus*

### Collection

#### *H. influenzae*

Specimens must be collected and cultured as soon as possible since the organisms do not survive well. Pure culture isolates from sterile sites such as blood or cerebrospinal fluid may be submitted to the ASPHL. It is not recommended that clinical materials be submitted to reference laboratories for isolation.

Note: Isolates should be transported on chocolate slants.

#### *H. aegyptius*

This organism is closely related to *H. influenzae*, and is the causative agent of contagious conjunctivitis. Conjunctival scrapings should be collected and cultured immediately. Pus may be collected on the tip of a calcium alginate swab and placed in a modified Stuarts Transport medium prior to culture. Reference isolates may be forwarded on to the ASPHL for confirmation.

#### *H. ducreyi*

Chancroid lesions should be carefully scraped or swabbed. These specimens should not be allowed to dry, and should be cultured immediately.

### Shipment of Specimens

Reference isolates should be transported on slants of chocolate or Levinthal agar. Both *H. aegyptius* and *H. ducreyi*, because of their fastidious nature, should be transported on chocolate agar slants supplemented with 1% IsoVitalEX.

See Section 8: Sample Submission Guidelines.

### Reporting and Interpretation of Results

*H. influenzae* serotype b has been identified as the leading cause of bacterial meningitis and epiglottitis. It has also been implicated as a major cause of pericarditis, pneumonia, septic arthritis, osteomyelitis, and facial cellulitis, as well as an occasional cause of urinary tract infection in children less than 5 years of age. Non-encapsulated strains may cause noninvasive respiratory infections in healthy children, and community acquired pneumonia and chronic bronchitis in adults.

Biotyping of *H. influenzae*, *H. parainfluenzae*, as well as the identification of other *Haemophilus sp.* is accomplished with biochemical testing.

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Serotyping is relevant only for the encapsulated strains of *H. influenzae* from sterile sites. Testing is performed for children under 5 years of age using a combination of rapid agglutination techniques in type-specific antisera, and PCR.

Serotype b is called to Bureau of Epidemiology and Disease Control.

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## *Legionella*

### **Collection**

Legionellae are most frequently isolated from specimens originating in the respiratory tract. On rare occasions, they may be isolated from extra-pulmonary sites including pericardial fluid, peritoneal fluid, wounds, and abscesses. Legionellae are not known to colonize humans, and therefore are not commensals of the respiratory tract. Respiratory secretions from those patients who are not able to provide adequate sputum specimens may be collected by transtracheal aspiration or bronchoalveolar lavage. On occasion, it may be necessary to collect lung tissue samples to establish the diagnosis of Legionnaires' Disease.

ASPHL no longer tests clinical samples for the routine isolation and identification of *Legionella*, isolates submitted for confirmation, or clinical samples to aid in outbreak surveillance. All clinical samples and isolates received by the ASPHL are forwarded to CDC. Sputum should be collected and transported in sterile containers with tight fitting lids. Use of saline in specimen collection fluids should be avoided, since sodium ions may be inhibitory to the organism.

### **Shipment of Specimens**

Special media are not required for transport of specimens, as long as they are protected from drying and rapid temperature changes. Specimens can be held at 4° C or transported on wet ice, provided they are examined within 48 hours of collection. Those that are to be held for longer periods should be stored frozen, preferably at -70° C and transported in the frozen state.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Cultures are forwarded to CDC for identification and serotyping. Positive cultures are called to the submitting client and to Bureau of Epidemiology and Disease Control.

## *Leptospira*

### **Collection**

Blood, cerebrospinal fluid (CSF), and urine are the specimens of choice for recovery of leptospires. The most appropriate choice is to culture during the first 10 days of illness, and to collect blood and CSF. The specimens should be collected prior to antibiotic treatment and while the patient is febrile. After the first week of illness, the optimal source for isolation of leptospires is urine.

If culture medium is not available, blood should be collected in tubes containing heparin or sodium oxalate. Tubes containing citrate should be avoided, since citrate may be inhibitory.

### **Shipment of Specimens**

Blood and CSF specimens should be stored and transported at room temperature (20°-30° C) and inoculated into culture medium within 48 hours of collection.

Urine should be inoculated within two hours, especially if the urine is acidic. **Media and instructions are available upon request for inoculation prior to submission to the ASPHL.**

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

All *Leptospira* cultures are forwarded to CDC.

Positive cultures are called to the submitting client and Bureau of Epidemiology and Disease Control.

## *Listeria*

### **Collection**

Clinical specimens from normally sterile sites such as blood, CSF, amniotic fluid, placenta, or fetal tissue do not require special procedures for collection or transport. Specimens from non-sterile sites, such as meconium, feces, and vaginal secretions, respiratory, skin or mucous swabs require prompt handling to prevent the overgrowth of contaminants.

Culture specimens from sterile sites can be plated directly to tryptic soy agar containing 5% sheep, horse, or rabbit blood. Samples for blood culture can be inoculated directly into conventional blood culture broth.

### **Shipment of Specimens**

Clinical specimens will be accepted only by prior approval of the Bureau of Epidemiology and Disease Control during an outbreak investigation. Specimens from sterile sites should be transported as soon as possible. If processing is delayed, specimens should be held at 35° C in an incubator for no longer than 48 hours. Specimens from non-sterile sites require prompt handling. If processing is delayed, the materials should be kept at 4°C or frozen at -20° C if testing delays are expected to exceed 48 hours. Ship at 4° C.

Non-sterile specimens (other than stool) can be stored at 4° C for up to 48 hours. For longer periods of storage, freezing specimens at -20° C is recommended.

Stools should be shipped frozen on dry ice.

Reference cultures can be transported on nutrient agar slants or other non-glucose containing agar slants at ambient temperature.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Inoculated media will be incubated for 72 hours and examined daily for growth. Isolates and reference specimens are streaked to a blood agar plate and examined daily for typical growth characteristics. Identification of *Listeria* is made based on colony and microscopic morphology and various biochemical reactions. The organism is then sent to CDC for serotyping.

Positive cultures are called to the submitting client and Bureau of Epidemiology and Disease Control.

## **Meningococcus** *Neisseria meningitidis*

### **Collection**

Specimens from which *Neisseria meningitidis* may be isolated include CSF, blood, petechial aspirates, biopsy samples, joint fluid, and conjunctival swabs. Only those isolated from sterile body sites are accepted at the ASPHL.

Inoculate specimens directly onto a nutritive, nonselective medium such as chocolate medium supplemented with IsoVitaleX or a blood agar medium and incubated in a CO<sub>2</sub> enriched atmosphere immediately after collection.

Isolates may be submitted on chocolate agar, Amie's, Stuarts or equivalent transport media.

### **Shipment of Specimens**

Transport reference isolates as quickly as possible to the ASPHL at room temperature. It is recommended that the containers be insulated during very hot or very cold weather. All cultures must be transported with minimum delay since viability is readily lost. If specimens must be transported from a distant town, the inoculated media must be incubated 18 - 24 hours before transport, and the specimen should arrive within 48 hours.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Cultures are examined daily for typical growth characteristics. Isolates are identified biochemically. *N. meningitidis* isolates are serotyped for epidemiological purposes using type-specific antisera.

Serogroup C results are called to the submitting client and Bureau of Epidemiology and Disease Control. Non-C serogroup results are called to Bureau of Epidemiology and Disease Control.

## Pertussis

### *Bordetella pertussis, B. parapertussis*

#### Collection

The specimen of choice for the recovery of *Bordetella pertussis* and *B. parapertussis* from the respiratory tract is secretions collected from the posterior nasopharynx. Specimens collected from the throat are not acceptable. Specimens collected from the nose are not optimal. Nasopharyngeal (NP) specimens may be collected as aspirates obtained by suction or perinasal swab specimens.

One or two perinasal swab specimens are collected by passing the swabs through the nares as far as possible into the nasopharynx. Leave the swab in place for up to 30 seconds. If resistance is encountered during insertion, try the other nostril. Rotate the swabs for a few seconds, and gently withdraw them.

**Use polyester NP swabs, swabs with cotton tips and wooden shafts are not permitted. Specimens collected with swabs made of calcium alginate are not acceptable as this material is inhibitory to the *Bordetella* spp. PCR. *Bordetella pertussis* is killed by the fatty acids found in cotton swabs.**

Push the swab, post collection, into a tube of Regan-Lowe semi-solid transport agar or Amie's with charcoal. When placing the swab into the tube break/snap/cut the shaft so that it fits properly into the tube. Swab shafts that are force or coiled into the tube may result in the sample being rejected as this creates that potential for laboratory contamination during PCR processing. Leave the swab submerged during transport to the laboratory.

**Only patients with signs and symptoms consistent with pertussis should be tested by PCR to confirm the diagnosis.** For guidance in distinguishing signs and symptoms of pertussis from those of other conditions, see <http://www.cdc.gov/pertussis/clinical/features.html>. Testing asymptomatic persons should be avoided as it increases the likelihood of obtaining falsely-positive results. Asymptomatic close contacts of confirmed cases should **not** be tested and testing of contacts should **not** be used for post-exposure prophylaxis decisions. Specimens submitted from asymptomatic contacts will be analyzed by culture only, in adherence to CDC recommendations PCR will not be performed.

**When possible, you should test patients for pertussis during the first 3 weeks of cough**, when bacterial DNA is still present in the nasopharynx. After the fourth week of cough, the amount of bacterial DNA rapidly diminishes, increasing the risk of obtaining falsely-negative results by PCR. For more information on diagnostic testing, see <http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-confirmation.html>

Also, PCR testing after 5 days of antibiotic use is unlikely to be of benefit, because PCR testing following antibiotic therapy also can result in falsely-negative findings, although the exact duration of positivity following antibiotic use is not well understood.

#### Shipment of Specimens

If possible, the cultures should be transported on ice. If transport to the laboratory is delayed, specimens should be refrigerated. Reference isolates of *B. pertussis* may be submitted to the ASPHL

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

A direct fluorescent antibody (DFA) test is not routinely performed on clinical specimens; identification is made based upon colonial morphology, microscopic appearance, and biochemical testing. Cultures are confirmed by DFA.

Positive culture and/or PCR results are telephoned to the submitting agency and to the Immunization Section of the Bureau of Epidemiology and Disease Control.

#### ***Salmonella***

See Enteric Culture (Page 1-5)

#### ***Shigella***

See Enteric Culture (Page 1-5). Reference specimens – For identification or confirmation only

### **Shiga Toxin**

#### **Collection**

Shiga Toxin

Stool specimens should be taken early in the course of illness when the causative agent is likely to be present in the largest numbers. Freshly passed stool is better than rectal swabs, and mucus and bloodstained portions can be selected for culture. Use a small amount of stool or rectal swab to inoculate a MacConkey (MAC) Broth or Gram Negative (GN) Broth and incubate at 35 – 37° C for 18 – 24 hours before submitting to ASPHL.

Transfer reference isolates of Shiga Toxin - producing organisms to a TSA or Nutrient Agar slant and forward to the ASPHL. A GN or MAC) broth may be submitted for isolation and confirmation of a toxin - producer.

#### **Shipment of Specimens**

GN or MAC broth may be submitted within seven days of inoculation, if stored and transported refrigerated (2-8 ° C). Cultures of pure isolates should be submitted within 48 hours of inoculation.

See Section 8: Sample Submission Guidelines.

## Reporting and Interpretation of Results

Stool samples, unless otherwise specified, will be screened for *E. coli* O157:H7 and other Shiga Toxin - producing organisms. Cultures are examined daily for 72 hours for characteristic morphology. Suspect colonies are screened biochemically, by EIA and/or PCR, and then confirmed with serologic agglutination (where applicable) or forwarded to CDC.

For outbreaks, all reports of Shiga Toxin - producing organisms are telephoned to the submitting agency and to the Bureau of Epidemiology and Disease Control.

## VISA / VRSA *Staphylococcus aureus*

### Collection

Submit a pure culture of a reference isolates for epidemiological studies or confirmation of VISA /VRSA (Vancomycin- Intermediate/Resistant *Staphylococcus aureus*)

### Shipment of Specimens

Isolates should be transported on a blood agar plate or slant. Transport specimens to the ASPHL at ambient temperatures.

See Section 8: Sample Submission Guidelines

## Reporting and Interpretation of Results

*Staphylococcus aureus* that have developed resistance to methicillin are referred to as MRSA; they are also resistant to most antibiotics commonly used for staphylococcus infections. These drugs include methicillin, oxacillin, nafcillin, cephalosporins, imipenem, and other beta-lactams. The infection is then generally treated with vancomycin. Most isolates of *S. aureus* are susceptible, but use of vancomycin can lead to the development of resistance as well. The minimal inhibitory concentration (MIC) of vancomycin required to inhibit these strains is typically  $\leq 2$  micrograms/mL ( $\mu\text{g/mL}$ ). In contrast, *S. aureus* isolates for which vancomycin MICs are 4-8  $\mu\text{g/mL}$  are classified as vancomycin-intermediate (VISA), and isolates for which vancomycin MICs are  $\geq 16$   $\mu\text{g/mL}$  are classified as vancomycin-resistant (VRSA). The revised definitions for classifying isolates of *S. aureus* are based on the interpretive criteria published in January 2011 by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). VISA isolates are not detected by disk diffusion. The ASPHL uses the E-test® using a 0.5 McFarland standard to prepare inoculum to confirm VISA/VRSA isolates before sending them to CDC.

All VISA/VRSA organisms are reported to the submitting agency and to Bureau of Epidemiology and Disease Control.

## *Vibrio*

### **Collection**

Stool specimens should be collected early, preferably within 24 hours of onset of illness, and before administration of antibiotics. Rectal swabs or fecal material should be placed in the semisolid transport medium of Cary-Blair, or Modified Cary-Blair. See Enteric Cultures (1-5)

### **Shipment of Specimens**

Specimens in transport media should be shipped to the ASPHL as soon as possible at ambient temperature. Isolates may also be submitted for serotyping or confirmation

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Cultures suspected to contain *Vibrio cholerae*, and other *Vibrio* species are tested with commercial biochemical systems. Cultures presumptively identified as *Vibrio cholerae* will be tested against specific antisera to determine the serogrouping of the isolate. *Vibrio cholerae* strains will fall into two groups based on this serological testing. O group 1 strains (O1) are associated with epidemic cholera; non-O1 strains may cause cholera-like and other illnesses, but are not involved in epidemics. *Vibrio cholerae* O1 strains are divided into three subtypes: Ogawa, Inaba, and Hikojima. The O1 strains are further divided into two biogroups: classical and El Tor.

Positive cultures of *Vibrio cholerae* are called to the submitting client and Bureau of Epidemiology and Disease Control.

## Section 2: Mycobacteriology

The ASPHL provides diagnostic and reference services for the isolation and identification of *Mycobacterium tuberculosis* (MTB) and other mycobacteria at no charge to all public and private health care providers in the state. The ASPHL receives a federal grant to support the statewide testing of *Mycobacterium* in support of the National Action Plan for the Elimination of Tuberculosis in the United States.

### Collection

Use a clean, sterile, leak-proof disposable screw-capped 50 mL conical centrifuge tube supplied by the ASPHL. Do not use waxed containers. More detailed information regarding how to obtain specimen collection and submitting materials can be found in Section 8: Sample Submission Guidelines and Section 9: Requesting Collection Kits and Mailing Containers. **Samples will not be processed for Mycobacteria if not received in the laboratory within 7 days of collection.**

### Sputum

In pulmonary tuberculosis and the related Mycobacterial diseases, sputum (phlegm from deep in the lungs) is the specimen of choice. A 5 - 10 mL sample of sputum is the desired volume for a single examination. Pooled specimens collected over several hours are not suitable for examination. A series of 3 early morning specimens, collected in clean sterile containers, on **consecutive days** should be obtained. Collect the initial specimens before antimicrobial therapy is started. Do not use fixatives or preservatives.

### Urine

The specimen of choice is a clean catch, midstream, first morning specimen. Urine should be collected in a clean, sterile, screw-capped plastic container. Pooled specimens or 24-hour urines are unacceptable. A series of first morning specimens should be collected on three consecutive days.

### Gastric Washings

Gastric washings are specimens of last resort because they are highly diluted with gastric fluid, which is damaging to the tubercle bacillus. Specimens should be delivered to the laboratory immediately so neutralization procedures can begin. **These samples are not suitable for mailing.**

### Specimens from Sterile Sites

These include cerebrospinal fluid (CSF), pleural fluid, ascitic fluid, joint fluid, pus, exudates, biopsy, and autopsy tissues. These are all surgical specimens and should be collected or taken by a physician or surgeon and placed in sterile containers. Tissue may be delivered in sterile saline. Do not add any preservatives. **Swabs are not optimal for the recovery of Mycobacteria. They are acceptable only if a specimen cannot be collected by other means. A comment will be added to the final report.**

## Shipment of Specimens

After collection, identify the specimen with the patient's name collection date AND collection time. Fill out the proper laboratory submission form, *Microbiology Submission Form* (located at [www.azdhs.gov/lab](http://www.azdhs.gov/lab)). Include the patient's name, date of birth, submitting agency, test request, and other pertinent demographic information.

Specimens should be refrigerated immediately after collection, prior to shipment. If specimens are to be shipped, it is necessary to place the specimen in a triple-packed mailing container to avoid contamination in the event of leakage. The desired mailing container consists of a primary screw cap collection tube, a secondary metal screw-capped container placed within a tertiary screw-capped cardboard outer mailer. These containers are provided by the ASPHL upon request. Place the submission form around the outside of the secondary metal container. Never place the form around the primary container. The triple-pack mailer is a safety requirement and a postal shipping mandate. Mail specimens as soon as possible after collection, to avoid overgrowth of contaminating bacteria.

Reference specimens may be submitted in tubed solid media or in a liquid culture medium, including Bactec, MGIT, MB-Bacti, and Septi-Chek. Reference specimens that are mailed or delivered by courier transport must be placed in a double mailing container. In the event of courier transportation, the specimen may be sent in a 50 ml conical centrifuge tube inside an inner metal container and then placed in a sealed plastic bag. Securely tighten all caps.

See Section 8: Sample Submission Guidelines

## Reporting and Interpretation of Results

Specimens are processed daily, five days (Monday – Friday) a week. Smears are examined daily by fluorescent microscopy, using a fluorochrome stain. The results of positive smears on all new patients are telephoned to the submitting agency within 24 hours from receipt with the exception of specimens received on Friday afternoons or on an afternoon prior to a holiday. Preliminary laboratory reports are prepared and sent out for all smear results.

Specimens are cultured onto both solid and liquid media. Cultures are examined for growth during a period of six weeks (on negative smears) and eight weeks for positive smears, before being reported as “No Mycobacteria isolated”. Cultures exhibiting typical colonial morphology are identified using High Performance Liquid Chromatography (HPLC). HPLC can be performed on cultures from both liquid and solid media. Allow 48 hours after detection of growth for identification of the organism. This method can be used to identify most known species of Mycobacteria.

Nucleic Acid Amplification (Cepheid GeneXpert MTB/RIF Molecular Assay) test is automatically performed on smear positive sputum specimens from new patients on samples processed at the ASPHL. The Cepheid GeneXpert MTB/RIF Molecular assay may also be performed on smear negative sputum specimens from new patients upon approval from the Arizona Department of Health Services, TB control section at (602)-364-0715. Submitters who process their own samples may also submit samples to the ASPHL for a Nucleic Acid Amplification (NAA) assay with the approval of the TB Control Section. Once approval has been given, the submitter must send a fresh sample to be processed by the ASPHL. If there is no other alternative but to use the sample

processed by the submitter, the report will be qualified with a disclaimer. Positive NAA test results are telephoned to the submitting agency within 24 hours from receipt with the exception of specimens received on Friday afternoons or on an afternoon prior to a holiday.

### **Drug Susceptibilities**

Drug susceptibilities are performed only on *Mycobacterium tuberculosis* (MTB) and *Mycobacterium kansasii*. If the MTB is resistant to any of the first-line drugs tested by MGIT, an indirect susceptibility is performed by the conventional agar proportion method, where an additional drug regimen is tested. Drugs tested by the MGIT method are isoniazid, rifampin, ethambutol and pyrazinamide. These results are reported within 17 days of MTB identification from culture for all initial diagnostic specimens. The agar proportion method includes the same drugs plus ethionamide, ofloxacin, and capreomycin. Taken together, final results for susceptibility is between three to six weeks from identification. Drug susceptibility testing of *M. kansasii* is performed by agar proportion method. Susceptibilities are performed every three months on specimens that remain positive for MTB and *M. kansasii*. MGIT susceptibility results are telephoned to the submitter. Susceptibility testing should be requested on subsequent isolates when a regimen appears to be failing. The manifestations of a failing regimen are: lack of conversion of smear and culture to negative within three months for persons receiving regimens containing both isoniazid and rifampin; lack of conversion of smear and culture to negative after five months for those receiving other regimens (without both isoniazid and rifampin); smears and cultures showing a decrease in number of organisms or colonies followed by a persistent increase in numbers ("fail and rise").

Note: When submitting MGIT samples for MTB drug susceptibility testing, indicate the date the MGIT instrument identified the sample as positive. Omitting this information will cause delays in susceptibility testing.

The results of all specimens are reported by mail to the submitter. In addition, all positive results are reported to the Tuberculosis Elimination Section of the Bureau of Epidemiology and Disease Control, Arizona Department of Health Services

### Section 3: Limited Parasitology

Intestinal and blood parasites are diagnosed mainly by morphologic examination of diagnostic stages of the microorganism. Properly collected and preserved specimens are of the utmost importance, since old or poorly preserved materials are of little value in establishing a diagnosis and may lead to erroneous conclusions. The ASPHL no longer accepts routine diagnostic samples. The laboratory offers screening for *Giardia* and *Cryptosporidium* to assist in outbreak investigations with approval from the Bureau of Epidemiology and Disease Control. All other submissions are forwarded to CDC with the approval of the Bureau of Epidemiology and Control.

#### Collection

##### Fecal specimens (*Giardia* and *Cryptosporidium*)

Collect the stool in a clean container or on clean paper, and then transfer to the Ova and Parasite (O & P) transport containers supplied by the ASPHL. The collection kit provided includes a container with PVA (polyvinyl alcohol) fixative and one container with 10% formalin fixative. Follow the instructions included with the containers. Mix thoroughly to assure adequate fixation. Do not contaminate specimen with urine or dirt. Administration of barium, magnesia, or oil before collection will render the specimen unsuitable for testing. Label each vial with patient's name and address. Because the host passes parasites intermittently, multiple specimens should be examined. These irregularities emphasize the need to collect at least three specimens over 10 to 14 days.

##### Blood Parasites

Blood smears are best made from blood not containing anticoagulants, since anticoagulants can interfere with parasite morphology and staining. For routine diagnosis, a thick film is preferable; however parasite morphology is more distinct and typical when observed in a thin film. Therefore, it is important to collect both thick and thin films for submission. Thin films are made by depositing a single drop of blood at one end of the slide and spreading it across the slide in preparation for a differential count. Thick films are prepared by touching the under-surface of a slide with a fresh drop of blood from a finger (without touching the skin) and rotating the slide to form a film about the size of a dime. Alternately, several drops of blood can be deposited at the end of a slide and puddle with an applicator stick or toothpick. Allow 8 - 12 hours drying time for a thick film before staining. Giemsa Stained slides should be placed in a cardboard slide holder, and labeled with proper identification.

If necessary, thick and thin smears can be prepared from anticoagulated blood, but the staining characteristics are not as good. EDTA anticoagulated blood is better for staining than citrate or heparin anticoagulant.

The time of specimen collection is important with malaria, but less important in other filarial infections. Malaria parasites are most numerous about midway between chills. One

specimen taken at this time and a second specimen collected 5 - 6 hours later is ideal. Because of nocturnal periodicity in filarial infections, the specimen should be taken between 10 PM and 2 AM. In *Loa loa*, there is diurnal periodicity, and these specimens should be collected between 10 AM and 2 PM.

### **Free Living Amoebae**

Unfixed specimens for culture (CSF or tissue) should be sent overnight at ambient temperature. Fixed specimens, including those in 70-90% ethanol, should be sent overnight on ice packs. Culture plates and slides must be packaged carefully to prevent breakage. All samples are forwarded to CDC for identification and/or confirmation.

### **Shipment of Specimens**

Fill out the *Microbiology Submission Form* which is located at <http://www.azdhs.gov/lab/shipping-receiving.htm>. Include the patient's name, date of birth, address, submitting agency, test request, and other pertinent information on the form. Identify the specimen with the patient's name and date of collection. Make sure that identification on the specimen matches the form. **Include travel history with the request for blood parasite identification.**

Specimens sent through the mail must be in containers that meet postal regulations for infectious materials. Specimen containers should be placed inside a double mailing container, which consists of an inner metal case with a screw cap placed within a screw-capped outer cardboard container.

Mailed stool specimens require use of one vial containing 10% formalin and a two-vial method of collection and shipping is advocated. **If you are submitting a stained slide for identification or confirmation, please include the original preserved sample for additional testing.**

See Section 8: Sample Submission Guidelines

### **Reporting and Interpretation of Results**

Specimens received for parasitology other than *Giardia* and *Cryptosporidium* will be forwarded to CDC with the approval of the Bureau of Epidemiology and Control. Blood smears forwarded on to the CDC for confirmation of results. A preliminary report will be generated by the ASPHL indicating that the specimen has been forwarded on to the CDC. The final report will be generated upon issuance of a report from the CDC.

### Section 4: Serology

The Serology Section is responsible for performing diagnostic testing for communicable diseases in support of outbreak investigations, and reference testing for private and public laboratories. The time required to process a serology specimen varies considerably, as indicated by the following table. Detailed information on the collection and submission of laboratory samples for any of the following tests can be obtained in the narrative guidelines that follow.

Agent	Specimen Required	Test Method	Reference Values	Turn Around Time (TAT) Business Days
Dengue <sup>2</sup> IgM	Serum	EIA	Negative	2-7 days
Chikungunya <sup>8</sup> IgM PCR	Serum	EIA PCR	Negative Negative	1-7 days
*Hantavirus <sup>2</sup> : IgG IgM	Serum	EIA	Negative	1-7 days
**Hepatitis C <sup>3</sup>	Serum	EIA	Negative	3-5 days
**HIV <sup>4</sup> :	Serum	4 <sup>th</sup> Gen. EIA Multispot	Nonreactive Negative	3-5 days 3-5 days
*Measles <sup>5</sup> Diagnostic: IgM	Serum	EIA	Negative	1-3 days
*Mumps <sup>5</sup> Diagnostic: IgM	Serum	EIA	Negative	1-3 days

Agent	Specimen Required	Test Method	Reference Values	Turn Around Time (TAT) Business Days
Rickettsial Panel <sup>7</sup> : Spotted Fever Group IgG Typhus Fever Group IgG Q Fever ( <i>Coxiella</i> ) IgG	Paired sera( acute and convalescent)	IFA	<64	2-14 days
*Rubella <sup>5</sup> Diagnostic: IgM	Serum	EIA	Negative	1-3 days
St. Louis Encephalitis <sup>1</sup> IgM	Serum, CSF	EIA	Negative P/N < 2.0	3-7 days
**Syphilis <sup>6</sup> : Screen	Serum	RPR	Nonreactive	1-5 days
Confirmation	Serum	TP-PA	Nonreactive	1-5 days
West Nile Virus <sup>1</sup> IgM	Serum, CSF	EIA	Negative P/N <2.0	3-7 days

\*Prior notification required

\*\* HIV programs only

Test abbreviations

EIA - Enzyme Immunoassay

IFA - Indirect Fluorescent Antibody

PCR – Polymerase Chain Reaction

RPR – Rapid Plasma Reagin

TP-PA - *Treponema pallidum* Particle Agglutination

1. Significant cross-reactions may be seen within the viruses in the Flavivirus group, including Dengue, St Louis Encephalitis (SLE) and West Nile Virus (WNV). If cross-reactivity is suspected, the sample will be forwarded to a reference laboratory for additional testing. IgM antibody may be absent if the specimen was collected too early in the course of infection. A convalescent serum sample should be submitted and tested if early collection is suspected.
2. Specimens submitted for Hantavirus testing are tested for both IgG and IgM antibodies. Demonstration of the presence of IgM antibody is suggestive of recent exposure to Hantavirus (Sin Nombre Virus). With prior notice and approval from the Arizona Department of Health Services Office of Infectious Diseases, the turnaround time for test results can be shortened.
3. Hepatitis C immune status testing is provided on a limited basis to the Arizona Department of Health Services HIV Program. Approval from the Arizona Department of Health Services Office of Infectious Disease is needed for any samples not qualifying for the ADHS HIV Program.
4. Samples submitted for HIV testing are tested by the 4<sup>th</sup> generation Enzyme Immunoassay (EIA) that will detect HIV antigen and HIV-1/HIV-2 antibodies in sera. Samples that test positive by EIA are retested in duplicate. Samples submitted for HIV testing are also tested by the HIV-1/HIV-2 Multispot rapid test. This testing algorithm follows the guidelines established by the Arizona Department of Health Services Bureau of Epidemiology and Disease Control and the Centers for Disease Control & Prevention.
5. The ASPHL provides only diagnostic testing for Measles, Mumps and Rubella. IgM antibody may be absent if the sera was collected too early in the course of infection, too late in the course of infection, or in the instance of disease due to vaccine failure. It is highly recommended that additional specimens be submitted along with the sera for alternative diagnostic testing methods for Measles, Mumps and Rubella (Refer to Section 5, Virology).
6. Syphilis testing is provided on a limited basis. Serum samples submitted for antibody testing for Syphilis are screened by the RPR, which is a non-treponemal test. Non-treponemal tests can be used for initial screening and for observing the patient's response to treatment. A non-reactive test may be interpreted as no current infection or an effectively treated infection. Non-treponemal tests will give a lower or non-reactive titer in the latent phase of infection.  

Samples that test reactive by the RPR are subjected to a confirmation test, by the TP-PA (*Treponema pallidum* Passive Agglutination). Use of this treponemal test should be reserved for confirming reactive non-treponemal tests, and for assisting in the diagnosis of late syphilis. Treponemal test misinterpretation often results from misuse of the treponemal tests as a screening procedure. About 1% of the general population has false-positive results with the treponemal tests.
7. Testing for Spotted Fever group rickettsia, Typhus Fever group rickettsia, or Q Fever will only be performed on paired sera. Single serum specimens may be tested but will require approval by the Arizona Department of Health Services Office of Infectious Disease.

Serum samples that cannot be tested or shipped to the ASPHL within 2-5 days of collection must be stored at  $\leq 20^{\circ}$  C in a non-self-defrosting freezer. Serum must be removed from the blood clot prior to freezing. Do not freeze blood specimens. With prior approval from the Serology department, acute serum samples can be stored at the ASPHL. Testing will proceed when the convalescent serum sample is received.

8. Chikungunya virus testing will consist of IgM EIA and PCR on all specimens received. During the first 8 days of illness, Chikungunya viral RNA can often be identified in serum, IgM antibodies are generally detectable after 8 days after onset of illness and can persist for months.

### Specimen Collection

For serological tests, 10 to 15 mL of blood should be collected aseptically in an appropriate collection tube. For serum red top, tiger top, gold top vacutainer tubes are acceptable. For whole blood or plasma specimens, a lavender top vacutainer tube with EDTA anticoagulant is acceptable. For pediatric patients, smaller volumes of blood may be collected in pediatric tubes. After collection, the tube may be transported directly to the Arizona State Public Health Laboratory (ASPHL) or the tube may be centrifuged and the serum/plasma poured off into a separate vial. The optimal volume of serum for routine submissions is 2 - 3 mL.

It is highly recommended that both acute and convalescent serum samples be run in parallel on the same test run looking for a rise in antibody titer. A four-fold rise in antibody titer between the acute and convalescent samples is indicative of a sero-conversion, indicating evidence of recent exposure to the microbial agent. The acute sample should be drawn as soon as possible after appearance of symptoms. The convalescent sample should be drawn 10 - 14 days after the acute sample.

Other specimens that may be sent to the ASPHL for serological testing include cerebrospinal fluid (CSF), pleural fluid, and joint fluid. Approximately 2 mL of sample is requested for testing. However, since these samples are difficult to obtain, all attempts will be made to test the samples if less than the ideal sample amount is submitted. **Store samples refrigerated and do not freeze. Submit on cool packs or wet ice.**

Samples may be considered unacceptable if they are grossly hemolyzed, contaminated with bacteria, lipemic, leak in transit, or are improperly labeled. Samples must be transported with the appropriate paperwork, verifying that the information appearing on the specimen matches that on the submission form. Since the integrity of the sample must be maintained from the time of collection of the sample until testing is completed, **labeling errors will result in rejection of the specimen.**

Laboratory submission forms should be filled out completely with all pertinent demographic information. Successful tracking of positive cases is reliant on complete and accurate information being supplied on these forms, including patient name or identifier, date collected, date of onset of illness, submitter's name and address, and agency code.

For HIV serological testing, specimens are to be submitted with an *HIV Submission Form* only. All other serological specimens should be accompanied with a *Microbiology Submission Form*.

## Shipment of Specimens

The specimen should be transported to the ASPHL as soon as possible. Due to the intense heat seen in the summertime, it is advisable to ship the specimens cold to prevent damage to the specimen in transit, or overgrowth with bacteria. Whole blood samples may be sent on cool packs, but should never be frozen. Freezing blood will cause lysis of the blood cells, and render the blood sample unsatisfactory for testing. Serum samples, if not tested within 2-5 days should be stored frozen at  $\leq 20^{\circ}$  C. and shipped to the ASPHL on ice. Specimens may be mailed or delivered by courier to the ASPHL.

See Section 8: Sample Submission Guidelines.

### If Sent by Courier

- Blood and blood products sent in vacutainer tubes should first be placed in a primary screw-cap leak proof container (such as a 50mL plastic conical tube available from the ASPHL) to reduce the risk of shattering while in transit.
- The specimen should then be placed in a secondary container such as a plastic specimen bag with separate compartments for the submission form and specimen.
- All infectious material must be triple packaged and conform to U.S. Department of Transportation (DOT) requirements.
- Pack the specimen and its form in absorbent material to help prevent breakage.

Note: It is acceptable to send more than one specimen together, as long as they are properly secured and identified.

### If Sent by Mail

- Blood sent in vacutainer tubes should first be placed in a leak proof primary container (such as a 50 mL conical tube available from the ASPHL) to reduce the risk of shattering while in transit.
- All infectious material must be triple-packaged and conform to current shipping regulations. Consult the Domestic Mail Manual published by the US Post Office (USPS) for current USPS requirements, and the Hazardous Material Regulations (HMR) for current DOT requirements.
- Wrap the submission form around the secondary container, and place inside the tertiary container or cardboard mailer. Package the specimen with enough absorbent material for entire contents and to help prevent breakage.

**Note: Do not put the submittal form around the primary container; it must be around the secondary container.**

- Place appropriate biohazard label on the outside of the secondary container before transportation to the ASPHL.

50 mL conical tubes and cardboard mailers are available from the ASPHL Receiving Section via request for materials form available at <http://www.azdhs.gov/lab/shipping-receiving.htm> and emailing to [labreceiving@azdhs.gov](mailto:labreceiving@azdhs.gov). Please submit your orders in advance to insure prompt service and delivery.

### **Specimen Rejection Criteria**

- Specimen not properly identified
- Identification on specimen does not match submittal form
- Broken in transit
- Leaked in transit
- Grossly hemolyzed, lipemic, turbid, or grossly contaminated
- No convalescent serum received
- Prior approval from the Bureau of Epidemiology was not given.

The submitter will be notified of all rejected laboratory specimens by telephone and with a laboratory report mailed to the submitting agency confirming the reason for rejection.

## Section 5: Virology

The Virology Section is responsible for performing diagnostic, reference, and surveillance testing for viruses. The time required to process specimens and render a final report may vary considerably depending upon the nature of the clinical material, the type of virus requested, and whether or not any virus is isolated in culture. The following table provides the viruses the ASPHL Virology department can identify and the turnaround times to report results. Detailed information on the collection and submission of laboratory samples for any of the following tests can be obtained in the narrative guidelines that follow.

Organism/Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT) Business Days
Adenovirus culture	Throat, Nasalpharyngeal, Eye	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		3-14 days
Arbovirus PCR Surveillance (WNV, SLE, Chikungunya)	Mosquito pools	None	Transport frozen	3-7 days
Cytomegalovirus (CMV) culture	Urine, Throat, N/P, Bronchial Wash, Biopsy, Whole Blood	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.	Urine should be transported within 24 hours (store at 4° C)	4 weeks
Enterovirus culture (including Coxsackie, Echovirus, and Polio)	Stool, Throat, N/P, CSF, Pericardial Fluid	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		3-14 days
Influenza PCR & culture • Seasonal	Throat swabs, N/P swabs, Nasal Aspirate, Nasal swabs, dual nasopharyngeal/throat	Hanks, Viral Transport Media (VTM), Universal Transport Media	Ship refrigerated or at -70°C if samples	PCR 1-7 days  Culture

Organism/Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT) Business Days
<ul style="list-style-type: none"> <li>Novel</li> </ul>	swabs, nasal wash, Bronchial wash, bronchoalveolar lavage, Tracheal Aspirate, Sputum, lung tissue	(UTM), Sterile Saline.	cannot be tested with 72 hours.	14-21 days
Measles (Rubeola) PCR & culture	<p>PCR: Buccal swabs are preferred. Nasal swabs, N/P swabs, N/P aspirates, throat swabs, urine and cell culture isolates in viral transport media are also acceptable.</p> <p>Throat, N/P, Urine, Whole Blood are acceptable for culture. The PCR specimens listed are also acceptable for culture.</p>	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		<p>PCR: 1-5days</p> <p>Culture: 7-14 days</p>
Miscellaneous Virus Culture	Misc. specimen types	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.	Contact ASPHL	7-14 days
Mumps PCR & Culture	<p>PCR: Buccal swabs are preferred but nasal swabs, N/P swabs, N/P aspirates, throat swabs, urine and cell culture isolates in viral transport media are also acceptable.</p> <p>Throat, N/P, Sputum, Urine, CSF, Buccal are acceptable for culture.</p>	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		<p>PCR: 1-5 days</p> <p>Culture: 7-14 days</p>

<b>Organism/Disease</b>	<b>Specimen</b>	<b>Transport Medium</b>	<b>Comments</b>	<b>Turn Around Time (TAT) Business Days</b>
	The PCR specimens listed are also acceptable for culture.			
Norovirus PCR	Raw stool	None	DO NOT FREEZE Contact Virology Lab before specimen submission	2-10 days
Parainfluenza type 1-4 culture	Throat, N/P, Sputum	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		7-14 days
Rabies	Small animal or animal head	None	See page 5-7	1-2 days
Rhinovirus culture	Throat, N/P	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		7-14 days
RSV Culture	Throat, N/P	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		7-14 days
Varicella-Zoster Culture	Vesicle Fluid	Hanks, Viral Transport Media (VTM), Universal Transport Media (UTM), Sterile Saline.		7-14 days

### Collection

In order to optimize the ability of the Virology Section to isolate and identify viral agents from clinical specimens, it is very important that the specimens be collected, handled and transported in a manner that minimizes deleterious effects on any viral agents present. In addition, sufficient information should be provided with a submitted specimen to guide the laboratory in the selection of proper inoculation techniques for the viral agents suspected.

### **Nasopharyngeal/Throat**

Virus isolation is most successful if respiratory specimens are collected within 3 to 5 days of onset of illness. Swabs from both the throat and nasal passage should be collected. The pharynx is swabbed vigorously with polyester or synthetic swab moistened with collection medium free of serum such as Hanks or Viral Transport Media (VTM), and then placed in a transport container containing Hanks or VTM. Break off the ends of the applicator sticks leaving the swab tips in the collection medium. Swabs with calcium alginate or cotton tips with wooden shafts are unacceptable for submission of specimens for viral culture.

NP swabs are used to collect specimens from the nasal passage. Allow the swabs to remain in the nasal passages for a few seconds to absorb the nasal secretions laden with virus. Place the swabs in the Hanks or VTM and label vial.

Store specimens frozen at  $-70^{\circ}\text{C}$  if they cannot be tested within 72 hours. Transport to the ASPHL on wet ice. Do not freeze at  $-20^{\circ}\text{C}$ .

### **Rectal**

Collect the specimen no later than 7-10 days after onset of illness. Use polyester, nylon or synthetic tipped swab moistened with Hanks or VTM to insert 4 – 6 cm into the rectum. Rub the mucosa until visible fecal material is present. Two swabs should be collected in this manner. Place the swabs into Hanks or VTM, and break the ends of the swabs. Freeze at  $-70^{\circ}\text{C}$  if specimens cannot be transported to the laboratory within 48 hours.

### **Urine**

Urine specimens are generally tested for Cytomegalovirus, although Measles, Mumps and Adenovirus can sometimes be found in urine. Collect the specimen as soon as possible after onset of illness. Clean voided specimens (10 – 20 mL) are collected in sterile containers and transported immediately to the laboratory on wet ice or cool packs. If urine is to be cultured for CMV, it must be transported to the laboratory as soon as possible, preferably within 24 hours. Specimens should be stored at  $4^{\circ}\text{C}$  and transported on wet ice or cool packs. **DO NOT FREEZE URINE FOR CMV.**

### **Throat Washings**

Throat washings should be collected by gargling with Hanks Buffered Salt Solution (HBSS). **DO NOT FREEZE SPECIMENS COLLECTED FOR ISOLATION OF RSV.**

### **Cerebrospinal Fluid (CSF)**

For virus isolation, 3 – 4 mL of CSF should be collected no later than 7 – 10 days after onset of illness. Place in a sterile screw capped tube without collection medium. If delays in transport, store frozen at -70° C. Transport to the laboratory on wet ice or cool pack.

### **Eye Specimens**

A polyester or synthetic swab moistened with sterile saline is used to collect secretions from the conjunctiva. Place the swab in the Hanks or VTM.

Scrapings from the cornea or conjunctiva should be collected by an ophthalmologist or trained physician and placed in Hanks or VTM.

### **Stools**

Place three to four grams of stool into a sterile container and transport to the laboratory on wet ice or a cool pack. Stool specimens collected to test for the presence of Norovirus must be refrigerated (not frozen) as soon as possible after collection.

### **Vesicular Lesions**

Vesicular fluids and cellular material from the base of lesions should be collected for virus isolation during the first three days of the eruption. The fluids should be diluted in 2 – 3 mL of Hanks or VTM virus collection medium to prevent clotting. Alternatively, the fluids may be collected on a polyester or synthetic swab and then placed into Hanks or VTM. Store refrigerated for up to 48 hours. If specimens are to be held for longer than 48 hours, store frozen at -70° C. Transport to the laboratory on wet ice or cool packs.

### **Blood**

Although blood is not the optimal specimen for isolation of most viruses, it may be used for the recovery of some of the vector-borne viruses, enteroviruses and CMV. Specimens for virus isolation should be collected as soon as a viral agent is suspected, otherwise early neutralizing antibody may prevent isolation of virus from the blood. Either serum or leukocyte preparations may be used for viral isolation. For isolation of virus from leukocytes, 8 mL of blood is collected in a tube containing an anticoagulant, preferably EDTA (heparin has been shown to inactivate Herpes virus and is inhibitory to PCR diagnostics that, when available, may accompany culture). For isolation of virus from the serum or blood clot, 8 mL of blood is collected aseptically without an anticoagulant. Transport on wet ice or a cool pack.

### **Autopsy or Biopsy Specimens**

Autopsy specimens should be collected within 24 hours after death. Samples from probable sites of pathology are collected using separate, sterile instruments and separate sterile

containers for each specimen. Tissues are transported to the laboratory on wet ice or cool pack. If they cannot be tested within 48 hours, they should be stored frozen at -70° C.

### **Shipment of Specimens**

All infectious material must be triple-packaged. Place specimens in screw-cap leak proof primary container. Place primary container in a leak proof secondary container and wrap submission form around secondary container. Place secondary container and submission form in an appropriate tertiary container. Ensure adequate ice or cool packs are used if required. Each specimen must be accompanied with a *Microbiology Submission Form*. Mail, ship or courier specimens to the ASPHL.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Specimens are read daily for typical cytopathic effect (CPE). Turnaround time for negative cultures varies from one to four weeks depending upon the viral syndrome suspected. Respiratory and enteric virus cultures are held for 2 weeks. Invasive respiratory specimens and CMV cultures are held for 4 weeks. Delays in reporting may be due to cultures that have one to several passages.

In addition, cultures yielding virus isolates may require more or less time for identification of the virus, depending upon the isolate involved. Failure to isolate a virus should not rule out a virus as a cause of clinical illness.

Influenza PCR samples must be shipped at -70° C if testing cannot be performed within 72 hours. A disclaimer will be added to all samples that states: "Specimens should be shipped at -70° C if testing cannot be performed with 72 hours of collection. If shipping conditions are not met, a negative test result does not rule out the presence of Influenza virus."

## Rabies

### Collection

Updated collection information can be found at <http://www.azdhs.gov/phs/oids/vector/rabies/>. The head of animals the size of dogs or smaller should be submitted. The head should be severed close to the shoulders allowing sufficient tissue of the throat to remain, to ensure inclusion of salivary glands. Prior approval from the Bureau of Epidemiology and Disease Control Vector Borne Diseases section is needed on all submissions. Contact them at 602-364-4562.

The brain of large animals, such as cows and horses, should be removed by a veterinarian and sent to the laboratory unless prior arrangements have been made.

Small animals such as bats, mice, rats and gophers may be sent intact.

**Please Note: Rodents will be tested only by prior approval from the Vector-Borne and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control.** Contact them at 602-364-4562 for instructions. Rodents may carry other serious and deadly diseases, such as Plague, Tularemia, or Hantavirus, and should be handled with extreme caution.

Birds and reptiles will not be accepted for examination.

Specimens for rabies examination should be collected immediately after the death of the animal. Decomposed specimens or specimens infested with maggots may not be testable but will be determined by the Virology Section.

### Shipment of Specimens

All infectious material including specimens submitted for rabies testing should be triple-packaged. Place the head in a leak proof primary container, and place the primary container in another leak proof secondary container. The secondary container and be placed in a tertiary container and filled with wet ice or cold packs as necessary. An animal ID should be written on the primary, secondary, or tertiary container to ID each sample submitted. An approved *Rabies Submission Form* should be placed in a separate sealed plastic bag outside of the secondary container, or in a separate plastic bag or envelope taped to the outside of the box. Ship the specimens to the Arizona State Public Health Laboratory in Phoenix. Testing delays may be experienced on specimens that are received frozen.

See Section 8: Sample Submission Guidelines.

### Reporting and Interpretation of Results

In all cases when exposure of a human is reported by a physician or veterinarian, laboratory examination will be made consisting of microscopic examination of smears prepared from brain material. The results of the microscopic examinations will be available 24 to 48 hours after receipt of the specimen. Positive results will be reported by telephone to the Vector-Borne and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control.

## Section 6: Environmental Microbiology

The Environmental Microbiology Section conducts microbiological examinations of food and water for sanitary quality and isolation and identification of microorganisms of public health significance. Sanitarians and representatives of federal, state, county and city agencies responsible for monitoring quality and enforcing regulations governing production and handling of food and water may, submit samples for analysis.

### Food Product Samples

In order to ensure rapid and efficient service, communication with the Environmental Microbiology Section is very important. Before submitting or shipping any samples for analysis, please call the Arizona State Public Health Laboratory (ASPHL) Environmental Microbiology Section (602) 542-6130.

A three-day food history and investigation observation should be used to guide the selection of appropriate foods for analysis. An investigation should be conducted before submitting samples to the lab for analysis.

#### Collection

After determining the appropriate food specimen to submit, aseptically collect approximately 200 grams of a solid product or about 100 mL of a liquid. Collection should be in a sterile whirl-pak plastic bag or sterile urine collection cup. The ASPHL does not provide sterile collection containers for food collection.

#### Shipment of Specimens

All samples must be kept cold ( $<10^{\circ}\text{C}$ ) during transit to the laboratory. Samples that are shipped should be placed in a leak-proof shipping container, preferably a Styrofoam container, packed with sealed cold packs (i.e. blue ice packs). Samples that are hand delivered on wet ice should be protected from cross contamination as the ice melts during transit.

See Section 8: Sample Submission Guidelines.

A properly completed *Food Analysis Submittal Form* must accompany **each individual sample**. Each sample must be identified by a unique number that corresponds to the identification number written on the submission form. More detailed information regarding how to obtain collection/submission supplies can be found in Section 9: Requesting Collection Kits and Mailing Containers.

#### Reporting and Interpretation of Results

Quality control samples are tested for aerobic plate count, total coliforms, fecal coliforms and *E. coli*. Pathogen isolation and identification is available for foods implicated in food borne illness outbreaks. Tests available include, but are not limited to, the following:

- *E. coli* O157:H7 detection and isolation
- *Salmonella* detection and isolation

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- *Listeria* spp. detection and isolation
- *Campylobacter* spp. detection and isolation
- *Bacillus cereus* plate count
- *Staphylococcus aureus* plate count
- Staphylococcal enterotoxins detection
- *Clostridium perfringens* plate count
- Container analysis
- Foreign object identifications
- Filth analysis

Food samples are analyzed according to methods specified in the Bacteriological Analytical Manual (FDA BAM) Microbiology Laboratory Guide (USDA MLG) by methods specified by the Centers for Disease Control and Prevention (CDC), or the Food Emergency Response Network (FERN). When appropriate, rapid analytical test kits are used to screen samples for pathogens to provide quicker test results during food outbreak investigations or emergencies. The rapid test results usually take only 1 to 2 days. However, positive results of these tests are only presumptive and conventional tests need to be done to confirm these results.

Preliminary results are usually available within 48 to 72 hours after processing has begun. Confirmatory test results are usually available within 48 hours to ten days depending on the test organism. Please contact the Environmental Microbiology Section at (602) 542-6130 at any time for updates on the progress of the testing. Generally, final reports are mailed out 3 to 11 days after initial processing begins.

Interpretation of lab results is the responsibility of the submitter. The laboratory will consult with the submitter, if requested. No legal food standards are available on most products, so care and common sense are needed in the interpretation of lab data. Use your experience and comparisons to evaluate the results.

## **Water Samples**

The laboratory no longer accepts routine water samples for microbiological analysis. Samples are accepted from the County Health Departments with prior approval from management. The laboratory tests drinking water for the presence of coliforms and *E. coli* in compliance with the Safe Drinking Water Act. In addition, the laboratory tests surface or source waters, wastewater and runoff waters for indicator organisms and occasionally pathogens. Please call the Arizona State Public Health Laboratory before submitting or shipping water samples for analysis.

### **Collection**

#### **Drinking Water Samples**

Drinking water samples should be collected in sterile four-ounce whirl-pak bags or sterile collection bottles with sodium thiosulfate added to neutralize any chlorine in the water. Aseptically collect water from the sample tap. If using sterile collection bottles fill to the 100 mL line and leave adequate air space. If using the whirl-pak bags, collect 125 mL of water. Be sure to whirl them closed tightly and tie the tabs together securely.

## Other Water Samples

Surface water, source waters, runoff waters, etc. can be aseptically collected in any appropriate size sterile whirl-pak bag or bottle (sodium thiosulfate is not needed); however, at least 125 mL is needed to test.

## Shipment of Specimens

### Drinking Water Samples

Drinking water samples must be received and tested within 30 hours of collection. For routine samples, it is recommended that samples arrive the first of the week. Samples may be mailed or sent by courier to the Arizona State Public Health Laboratory to arrive the next day. While drinking water samples do not need to be iced during transit, it is recommended when feasible to cool samples. Each sample must be accompanied by a properly completed *Water Microbiological Sample Submission Form*. Information regarding how to obtain collection/submission supplies can be found in Section 9: Requesting Collection Kits and Mailing Containers. The form is also located on the website <http://www.azdhs.gov/lab/shipping-receiving.htm>.

### Other Water Samples

These waters need to be received in the laboratory within six hours of collection, and must be iced during transit. Since the transit time is so short, it is usually best to send the water samples to the laboratory by courier. A properly completed *Water Microbiological Sample Submission Form* must accompany each sample. More detailed information regarding how to obtain collection/submission supplies can be found in Section 9: Requesting Collection Kits and Mailing Containers. Before submitting these water samples, please call the Environmental Microbiology Section at (602) 542-6130 to arrange for testing.

See Section 8: Sample Submission Guidelines.

## Reporting and Interpretation of Results

### Drinking Water Samples

Drinking water samples are routinely tested for the presence of total coliforms and *E. coli* using the enzyme substrate coliform test. This method provides results in 18 to 24 hours. This is the EPA approved method SM 9223B.

Results of drinking water coliform tests are usually available within 18 to 24 hours after processing has begun. All positive results are called to the submitter, providing that a telephone number has been supplied. In addition, all compliance positive results and repeat samples are faxed to ADEQ (Arizona Department of Environmental Quality). Leaked in transit and too long in transit samples are also called to the submitter. Final reports will usually be mailed one to two days after initial processing. If the sample is checked as a compliance sample, a copy is sent to the submitter and ADEQ.

Normally, the maximum contaminant level for total coliforms in drinking water is based on the presence or absence of coliform organisms in a 100 mL sample. A single water sample

can have 0 coliforms per 100 mL. Other rules apply when more routine samples are collected, as the ADEQ compliance Department dictates. The number of samples required is based on the population served by a public water system. If a compliance sample is positive, repeat samples need to be collected. Please contact your ADEQ compliance officer to determine the number and location to collect these repeat samples.

### Other Water Samples

Other types of waters are tested for indicator organisms such as fecal coliforms, *E. coli*, fecal *Streptococcus* and *Enterococcus* using either a Most Probable Number (MPN) method or a Membrane Filter (MF) method. The methods are Standard Methods. (A list of methods is outlined in the table below). On occasion, waters are tested for pathogens, such as *Salmonella*. Please contact the Environmental Microbiology Section for these requests.

Method Name	Units	Standard Method Number	Holding Time	Matrix	Temp °C
Presence/Absence (PA) Coliform Test	Presence or Absence/100 ml	SM 9221D	30 Hours	Drinking, Well or Ground Water	Ambient
Enzyme Substrate Coliform Test (Colilert/Colisure)	Presence or Absence/100 ml	SM 9223B	30 Hours	Drinking, Well or Ground Water	Ambient
Colilert MPN - Most Probable Number (QuantiTray - MPN)	MPN Index per 100 ml	SM 9223B	8 Hours	Surface/Ambient and Wastewater	< 10 °C
Fecal Coliform Membrane Filter (MF)	C.F.U./100 ml	SM 9222D SM 9221E	8 Hours	Surface/Ambient and Wastewater	< 10 °C
Multiple Tube Fermentation Method (15 Tubes - M.P.N.)	MPN Index/ 100 ml	SM 9221B SM 9221E	8 Hours	Surface/Ambient and Wastewater	< 10 °C
<i>E. coli</i> Determination (E.C. broth with MUG)	C.F.U./100 ml or MPN Index/100 ml	SM 9221F	8 Hours	Surface/Ambient and Wastewater	< 10 °C
Heterotrophic Plate Count – HPC	C.F.U./ml	SM 9215B	8 Hours	Drinking water	< 10 °C

- Holding time of 30 hours for drinking water is the time of collection to start of incubation
- Holding time of 8 hours for surface/ambient and wastewater is time of collection to time of test start.

Other waters and their testing results are usually available within 1 to 5 days, depending on the method used and the target organism. Call the Environmental Microbiology Section at (602) 542-6130 for an update at any time. Final reports are mailed to the submitter when all tests are completed. The significance of the results of other waters and their tests depends on the circumstances. Consult with the Arizona State Public Health Laboratory and ADEQ if needed.

## Section 7: Bioemergency Detection and Response (Select Agents)

Since the terrorist events of September 2001, the ASPHL has set guidelines for the submission of miscellaneous powders and other suspicious substances for detection of priority biological agents (i.e., anthrax, plague, etc.). Additionally, clinical specimens of patients exposed to an intentional release of these priority biological agents, as well as patient specimens in association with naturally occurring infection, may be submitted for the organisms listed below. Notification of molecular results for the appropriate agent(s) will occur by telephone, usually with-in 2-5 hours of test completion followed later with final results based on organism recovery and biochemical assessment.

The Arizona Department of Health Services, State Public Health Laboratory is the only Laboratory Response Network (LRN) reference laboratory in the State. As an LRN reference laboratory utilizing CDC developed procedures and testing algorithms, we have the ability to provide confirmatory rule-in/rule-out service for the priority biological agents listed in Table 1 below. Given that a number of the organisms listed below have low (10-100 organisms) infectious doses we ask that extreme caution be taken when handling specimens suspected of containing any of these organisms. *Brucella spp.* and *F. tularensis* are consistently listed as some of the most commonly reported laboratory-associated bacterial infection.

Please contact the State Bureau of Epidemiology and Disease Control at (602) 364-3676 (main number) or (480) 303-1191 (after hours number) and the Bioemergency Detection and Response Laboratory at (602) 364-0999 before submitting samples for potential outbreak or unusual suspect organisms. In the event that an intentional release of any biological agent is suspected, contact your local county health department, local law enforcement agencies, and the FBI Phoenix field office at (602) 279-5511 to inform them of the incident.

### Specimen Collection and Shipping

Collected samples, clinical (Table 1) or environmental (Table 2), should be sent to the Arizona State Public Health Laboratory for testing as soon as possible to ensure the reliability of test results and/or to maximize the potential for recovery of viable organisms where appropriate. Sample collection should be consistent with current medical practices for the disease/organisms biology. In addition the specimen type should be based on the desired organism identification and test type to be performed (see Table 1). All clinical samples must have the following information on the submission form: Patient name, birth date, date and time of collection, sample source, contact information and test request.

Clinical samples, or reference isolates, should be submitted when a high suspicion exist that one of the organisms listed in Table 1 may be the cause of clinical disease (based on clinical signs, symptoms and exposure history) or when a recovered isolate from clinical or environmental samples cannot be ruled out based on traditional microbiological biochemical reactions. Additional information about appropriate clinical specimens and shipping conditions can be found on the ASM website listed below: <http://www.asm.org/index.php/issues/sentinel-laboratory-guidelines>

Environmental specimens should be of sufficient quantity and may consist of food, soil, smooth non-porous surfaces swab/wipe, powders, or packages (Table 2). Regardless of the sample type/source, collection should be performed by an individual with the knowledge and technical abilities to collect such a sample (i.e. food samples collected by trained sanitarian, powder samples collected by HAZMAT technician) and should be consistent with current practices for such sample. Prior to submitting environmental samples, contact the Bioemergency Detection and Response testing laboratory section (602)364-0999) for submission guidance and approval. For details regarding the collection and submission of powder samples/suspicious unknowns including those associated with a threat, please contact your local law enforcement and refer to the Arizona Department of Health Service Suspicious Substances guidelines:

<http://www.azdhs.gov/phs/emergency-preparedness/documents/response-plans/guidline-suspicious-substance.pdf>

All organisms (in culture form) listed in this section are considered Category A infectious substances and must be shipped accordingly. For information regarding the packaging and shipping of Category A infectious agents please refer to “Section 8: Sample Submission Guidelines” of this manual and/or the ASM Packaging and Shipping Guidelines for Sentinel Laboratories <http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf>.

### Sample Requirements

<b>Organism / Disease</b>	<b>Specimen Type</b>	<b>Transport media</b>	<b>Turnaround Time*</b>
<b><i>B. anthracis</i></b> (Anthrax)	<b>PCR:</b> Whole blood**, serum, isolate (liquid or plated), plasma, pleural fluid, transtracheal aspirate, bronchial lavage, sputum, CSF, clinical swabs  <b>Organism isolation:</b> vesicular fluid, swab of eschar material, blood, sputum, stool, CSF	Whole blood with EDTA**  Standard bacterial transport media	Clinical specimen: 4 Days  Reference isolate: 3 days
<b><i>Brucella spp.</i></b> (Brucellosis)	<b>PCR:</b> Whole Blood** (200 µl min) Serum (200µl min) Isolate (liquid or plated)  <b>Organism isolation (human):</b> Blood**, bone marrow, spleen, liver, abscess fluid, CSF, joint fluid <b>Organism isolation (animal):</b> Blood**, supramammary lymph nodes, uterus, ovary, mandibular lymph node, kidney, liver, bladder	Whole blood with EDTA  Standard bacterial transport media	Clinical specimen: 20 Days  Reference isolate: 11 days
<b><i>Burkholderia spp.</i></b> (Glanders– <i>B. mallei</i> ) (Meliodiosis– <i>B. pseudomallei</i> )	<b>PCR:</b> Whole Blood** (200 µl min) Serum (200 µl min) Isolate (liquid or plated)  <b>Organism isolation:</b> Blood**, bone marrow, urine, abscess aspirates, tissue biopsies, sputum, wound swabs	Whole blood with EDTA  Standard bacterial transport media	Reference isolate: 10 Days  Reference isolate: 7 days

<b>C. Burnetii</b> (Q Fever)	<b>PCR:</b> Whole Blood** (200 µl min)	Whole blood with EDTA	3 Days
<b>F. tularensis</b> (Tularemia)	<b>PCR:</b> Whole Blood** (200 µl min) Isolate (liquid or plated)  <b>DFA:</b> isolate (liquid or plated), ulcer swab, aspirate, tissues (lymph node or lung), bronchial/tracheal wash, pleural fluid, sputum, Autopsy/necropsy specimens (abscess material or sections of lymph node, lung, liver, spleen or bone marrow scrapings)  <b>Organism isolation (human):</b> Blood**, tissue biopsy (Lung or lymph node), aspirates (lymph node), ulcer swab, bronchial/tracheal wash, pleural fluid, sputum <b>Organism isolation (animal):</b> Aspirates (i.e. e., lymph node), Necropsy specimens (abscess material or sections of lymph node, lung), liver, spleen or bone marrow scrapings	Whole blood with EDTA  Standard bacterial transport media	Clinical specimen: 10 Days  Reference isolate: 5 days
<b>Orthopox</b>	<b>PCR:</b> Dried vesicle fluid on a slide, fresh biopsy, skin or crust from roof of vesicle, swab of lesion (dry or wet), fresh biopsy of pustule or vesicle (no formalin)	dry swab is preferred (no viral transport media) Store samples at 2-8° C	3 Days
<b>Y. pestis</b> (Plague)	<b>PCR:</b> Isolate (liquid or plated), bronchial wash, transtracheal aspirate, sputum, nasopharyngeal swab  <b>DFA:</b> Lymph node aspirate or smear, tissue biopsy (lymph node or lung), blood in blood culture bottle, bronchial/tracheal wash, isolate, Autopsy/necropsy specimens (abscess material, lymph node, lung, liver, spleen, and/or bone marrow scrapings)  <b>Organism isolation (human):</b> Aspirates (i.e. lymph node), biopsy of affected area (e.g. lymph node, lung), bronchial wash, transtracheal aspirate, sputum, blood, autopsy specimens: abscess material or sections of lymph node, lung, liver, spleen. <b>Organism isolation (Animal):</b> Aspirates (i.e. lymph node), Necropsy specimens (abscess material or sections of lymph node, lung, liver, spleen or bone marrow scrapings)	Whole blood with EDTA  Standard bacterial transport media	Clinical specimen: 10 Days  Reference isolate: 5 days
	Pure isolates or primary growth plates may be submitted for rule-out of the above mentioned organisms.		

\*Indicates time when confirmation results are available

\*\* Blood samples should be submitted to the laboratory in tubes containing EDTA. Other anticoagulants such as heparin, citrate and EDTA are acceptable for culture only as they do not inhibit the viability of bacteria. Heparin has been shown to inhibit PCR. Blood samples submitted in heparin containing tubes will not be tested via PCR due to the known inhibitory effects of this substance.

Table 2 – Environmental specimens		
Specimen Type	Amount	Notes
Soil	50g – 1000g	Collect 1” to 2” of surface soil.
Smooth non-porous Surface (counter, instrument, etc.)	26 cm <sup>2</sup> (4 inch <sup>2</sup> )– Macrofoam swabs 645 cm <sup>2</sup> (100 in <sup>2</sup> ) – Cellulose sponge-wipe	Use sterile swab or Cellulose sponge-wipe. Synthetic fibers, synthetic or metal shafts strongly preferred.
Powder	Up to 5g	Collect aseptically.
Food <sup>a</sup>	25 - 100g	If food is not available, submit empty containers. Food must be suspected of being intentionally contaminated with one of the agents listed below.
Isolate <sup>a</sup>	Isolate streak or slant and Original isolation plate	Send in both plates or tubes.

<sup>a</sup> Refrigerate immediately and transport on ice. Keep good records and send the Arizona State Public Health Laboratory a copy.

## Results Reporting

The results of clinical and environmental samples will be reported to the submitter and all other relevant agencies in a manner (phone notification or electronic messaging) and time frame consistent with the submitted sample type and the desired organism identification algorithm. For questions regarding sample submission, turn-around-times or reporting mechanisms please contact the Bioemergency Detection and Response laboratory section at (602)364-0999.

Following the detection and confirmation of *B. anthracis*, *Brucella spp.*, *Burkholderia spp.*, *C. Burnetii*, *F. tularensis*, *Y. pestis* or other Select Agents or Toxins ([www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html](http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html)), it is a requirement of the Select Agent Program that sections of the APHIS/CDC Form 4A (Report of the Identification of a Select Agent or Toxin) be submitted by both the laboratory confirming the identification and the laboratory submitting the specimen for identification. When a select agent is identified by ASPHL, the submitting laboratory will be contacted by ASPHL with the information regarding the need to fill out and submit the completed sections C and D of the APHIS/CDC Form 4A to CDC.

## **Anthrax** ***Bacillus anthracis***

Members of the *Bacillus* genus are aerobic, gram-positive spore forming bacteria. *Bacillus anthracis* is the causative agent of anthrax.

### **Collection**

For cutaneous anthrax, sterile swabs are appropriate for collection of vesicular fluid and eschar material. Vesicular fluid should be obtained from a previously unopened vesicle(s) using a sterile swab. To collect eschar material, carefully lifting the eschar's outer edge, insert a sterile swab and slowly rotate for 2-3 seconds beneath the edge of the eschar without removing it. Place the swab in the appropriate transport container and ship at 2-8°C.

If intestinal or pulmonary anthrax is suspected, blood, serum, plasma, pleural fluid, transtracheal aspirates, sputum, fresh or frozen tissue, stool or rectal swab can be submitted for culture and /or PCR (refer to Table 1 above for appropriate specimen-test combinations).

### **Shipment of Specimens**

*Bacillus* species are hardy and usually survive transport to the ASPHL either in freshly collected specimens or in a standard transport medium. Specimens should be shipped/transported at 2-8°C if they will not arrive at the ASPHL within 1 hour of collection. Reference isolates may be submitted on agar slants.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Cultures are incubated for 72 hours and checked daily for characteristic macroscopic morphology. Suspected isolates are tested biochemically and by Real-Time PCR. Confirmation is made by using LRN protocols and algorithms. Once a *Bacillus* isolate has been confirmed as negative for anthrax, any further identification will require approval from the Technical Supervisor.

All results, positive or negative will be phoned to the submitting agencies and when appropriate, reported to the Bureau of Epidemiology and Disease Control and local and federal law enforcement.

## **Brucellosis** ***Brucella spp.***

*Brucella spp.* are small, non-motile gram-negative coccobacilli that are pathogenic to humans and animals. *Brucella spp.* are usually transmitted to humans by direct contact, consumption of contaminated food products, or inhalation. *Brucella* is one of the most common causes of laboratory acquired infections

### **Collection**

Specimens that can be collected and cultured for the isolation of *Brucella* include blood\*, bone marrow, abscess fluid spleen and liver biopsies, cerebrospinal fluid (CSF) and joint fluid. Additionally, recovered isolates from clinical specimens and whole blood may be submitted for PCR analysis. Environmental samples such as food and water may be submitted.

\*When brucellosis is suspected, multiple blood cultures should be obtained.

### **Shipment of Specimens**

Specimens should be cultured as soon as possible after collection or refrigerated if delays are unavoidable.

All specimens should be kept at refrigerated temperatures during shipment.

Reference isolates may be submitted on agar slants

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Cultures for *Brucella* are held for 20 days, and are checked daily for typical growth. Suspected isolates are examined by Gram staining for typical microscopic morphology. Identification, confirmation and speciation are made through biochemical testing and Real-Time PCR.

All results, positive or negative will be phoned to the submitting agencies and when appropriate, reported to the Bureau of Epidemiology and Disease Control and local and federal law enforcement.

## **Melioidosis and Glanders** ***Burkholderia spp.***

Members of the genus are slightly curved gram-negative bacilli. *B. mallei* is the causative agent of Glanders and *B. pseudomallei* the causative agent of Melioidosis. Laboratory-acquired infections have been documented, thus all patient specimens and culture isolates suspected of containing/being either *B. pseudomallei* or *B. mallei* should be handled while wearing gloves and a gown. If either of these organisms is suspected further work should be done in a BSL-3 setting or at minimum BSL-2 facilities with BSL-3 practices.

Laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the ASPHL.

### **Collection**

Specimens that can be collected and cultured for the isolation of *B. mallei* and *pseudomallei* include blood, urine, sputum, abscesses and tissue aspirates. For PCR analysis, submit whole blood, serum, or isolates (pure cultures or primary growth plates).

### **Shipment of specimens**

Specimens should be cultured as soon as possible after collection or refrigerated if delays are unavoidable.

All specimens should be kept at refrigerated temperatures during shipment.

See section 8: Sample submission guidelines

### **Reporting and Interpretation of Results**

Cultures for *Burkholderia spp.* are held for 7 days and checked daily for typical growth. Suspected isolates are examined by Gram staining for typical microscopic morphology. Identification is made by Real-Time PCR and biochemical testing. Positive isolates of *B. mallei* or *B. pseudomallei* may be forwarded to the Centers for Disease Control and Prevention in Atlanta, Georgia for confirmation of laboratory results.

All results, positive or negative will be phoned to the submitting agencies and when appropriate, reported to the Bureau of Epidemiology and Disease Control and local and federal law enforcement.

## Orthopoxvirus

Orthopoxviruses are one of 8 genera that comprise the Poxviridae family of viruses and includes viruses such as variola virus (Smallpox), vaccinia virus (Smallpox vaccine), and monkeypox. The ASPHL conducts testing to detect the presence of orthopoxviruses as a means to rule-out the possibility of Smallpox.

### Collection

**Prior to sample collection and shipment**, if Smallpox is suspected a **mandatory** CDC Risk Assessment algorithm must be completed by Arizona State Department of Health Services personnel. In order for the department to evaluate each individual case per the CDC algorithm; contact William Slanta (602) 542-6128 at the ASPHL with a list of relevant clinical symptoms and complete patient information including vaccination and travel histories.

If testing is to be conducted at the ASPHL, the following samples may be submitted for testing: vesicular fluid, skin or crust from the roof of a vesicle, nylon swab of a lesion, or fresh tissue biopsy (Submit swabs, biopsy tissue and scabs dry, DO NOT add viral transport medium, a dry swab is preferred.).

Caution should be used when collecting clinical specimens thought to contain Smallpox. All processes including collection, processing, and packaging and shipping should be performed using BSL-2 (or BSL-3 if available) practices. The individual collecting the sample should wear the appropriate personal protective equipment including gloves, disposable gown, shoe covers, mask and eyewear or face shield. Respiratory protection is not necessary, but is recommended for individual with recent vaccination.

Contact the Bioemergency Detection and Response Laboratory (602) 364-0999, for details or questions regarding the specimen collection process.

### Shipment of Specimens

If upon completion of the risk assessment it is decided that the sample meets the CDC criteria for Smallpox testing, the Arizona State Department of Health Services State Public Health Laboratory will either accept and test the sample or forward the specimen to a laboratory with the appropriate safety level facilities for testing.

Package specimens from each individual being tested separately, **do not** package samples from multiple patients in one bag. Samples should be shipped within 24 hours of collection and be held at 2-8°C. If samples will not be received in the lab within 24 hours, samples should be stored and shipped on dry ice or at -20°C to -70°C. All packages must meet the current IATA and DOT standards for shipping infectious substances.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

Specimens submitted for Smallpox testing will be tested for the presence of orthopoxvirus and non-variola orthopoxvirus DNA by PCR. Testing conducted at the ASPHL detects the presence of orthopoxvirus DNA but does not exclusively detect the presence of Smallpox DNA.

All results will be reported (via phone call) to the submitting agencies and the Bureau of Epidemiology and Disease Control. Positive results will be reported to Centers for Disease Control and Prevention. Positive sample material may be forwarded to the Centers for Disease Control and Prevention in Atlanta, Georgia for additional laboratory testing.

Uncontrolled Copy

## **Plague**

### ***Yersinia pestis***

*Yersinia spp.* are non-spore-forming, gram-negative, coccobacilli or rod shaped bacilli. *Yersinia pestis* is the causative agent of the Plague. Plague can present in one of three clinical manifestations: bubonic, septicemic or pneumonic. Initially humans are infected with the bite of an infected animal or a flea. Once bitten, the affected individual could develop bubonic or septicemic plague from one of these bites. This disease development could occur with or without the development of pneumonic plague. If the disease progresses to the lungs, patients can transmit *Y. pestis* via aerosols to others. Patients with plague often develop necrotic lesions in the peripheral blood vessels, which can give the skin a black color, thus the name “black death”.

#### **Collection**

Clinical samples that may be submitted to the laboratory for identification of *Yersinia pestis* include aspirates (i.e. lymph node), biopsy of affected area (e.g. lymph node, lung), sputum, bronchial wash, transtracheal aspirate, blood, and sputum. Autopsy specimens include: abscess material or sections of lymph node, lung, liver, spleen. Consult Table 1 for specimen-test combinations on page 7-3.

Animal specimens may also be sent for isolation and identification procedures. Lymph node abscess material and necropsy specimens including: (abscess material or sections of lymph node, lung, liver, spleen or bone marrow scrapings may be used for animal submissions.

#### **Shipment of Specimens**

Transport samples to the ASPHL.

Tissue samples such as buboes, lung or lymph nodes should be collected into a sterile container. For small samples, add 1-2 drops of sterile normal saline to keep the tissue moist.

See Section 8: Sample Submission Guidelines.

#### **Reporting and Interpretation of Results**

Cultures are identified by observing typical colonial morphology. Typical colonies are presumptively identified using a Direct Fluorescent Antibody (DFA) test and/or Real-Time PCR. A positive DFA or PCR test is considered presumptive positive for Plague. All presumptive positive results are telephoned to the submitting agency and to the Vector Borne and Zoonotic Disease Section of Epidemiology and Disease Control. Cultures suspected of containing Plague are tested and confirmed using conventional biochemicals.

Cultures are held for 7-10 days before reporting as negative.

## **Q Fever** ***Coxiella burnetii***

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a species of bacteria that is distributed globally. Cattle, sheep, and goats are the primary reservoirs of *C. burnetii*. *C. burnetii* is an intracellular bacterium that must be grown in cell culture.

### **Collection**

Specimens that can be collected and tested by the ASPHL are whole blood in EDTA and environmental swabs.

### **Shipment of specimens**

Specimens should be collected as soon as possible and refrigerated if delays are unavoidable.

All specimens should be kept at refrigerated temperatures during shipment.

See section 8: Sample submission guidelines

### **Reporting and Interpretation of Results**

Whole blood and swabs are tested by Real-time PCR for presence of *C. burnetii* DNA. Positive samples of *C. burnetii* will be reported and may be forwarded to the Centers for Disease Control and Prevention in Atlanta, Georgia for confirmation of laboratory results.

## **Tularemia** ***Francisella tularensis***

The genus *Francisella* are tiny aerobic non-motile gram-negative coccobacilli. *Francisella tularensis* is the causative agent of Tularemia, a disease that can be misdiagnosed early in the infection because the symptoms are not unique. Humans typically acquire *F. tularensis* after contact with tissues or body fluids of infected animals or an insect bite.

### **Collection**

During infection, direct isolation is achieved from ulcer scrapings/swabs, lymph node biopsies, bronchial/tracheal washings, sputum, and pleural fluid. In human cases, several sources should be considered. Organisms are invariably present in significant numbers in fluid from obvious local lesions. Skin around the lesion should be cleansed with alcohol and allowed to dry before opening the papule and exposing the fluid. Organisms may persist for long periods of time in lymph nodes and may be isolated by node biopsy. Consult Table 1 for specimen-test combinations.

### **Shipment of Specimens**

Transport samples to the ASPHL.

See Section 8: Sample Submission Guidelines.

### **Reporting and Interpretation of Results**

*F. tularensis* requires cystine enriched medium for growth. The historic medium of choice is cystine glucose blood agar; however any media containing cystine supplementation is acceptable. Cultures are observed for 7-10 days before reporting as negative. Cultures are observed for typical colonial morphology. Suspect colonies are checked microscopically by Gram staining, where they appear as faintly staining gram-negative coccobacilli. Confirmation of the isolate is determined by Direct Fluorescent Antibody, and Real-Time PCR.

All positive cultures are reported to the submitting agency and the Vector Borne and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control. *F. tularensis* isolates are forwarded to the Centers for Disease Control and Prevention at Atlanta, GA. upon request.

## Section 8: Sample Submission Guidelines

Submit all samples to the following location:

Arizona Department of Health Services  
State Public Health Laboratory  
250 N. 17<sup>th</sup> Ave  
Phoenix, AZ 85007  
(602) 542-1188

All infectious material must be classified as either Category A (UN2814) or B (UN3373) and must be transported to the Arizona State Public Health Laboratory according to appropriate IATA (International Air Transportation Association), USPS (United States Postal Service) and DOT (U.S. Department of Transportation) regulations. The list of Category A organisms as outlined by IATA is contained at the end of this section. All infectious material must be triple-packaged to protect against breakage and/or leakage during transportation. An ASPHL Submission Form must accompany every sample submitted for testing, the form is available at: <http://www.azdhs.gov/lab/shipping-receiving.htm>. Category B shipments must follow Packaging Instruction (PI) 650.

Category A shipments must follow PI 620 and shipments with dry ice must follow PI 954. All samples and their containers must be identified with the appropriate labels, client and patient information. Any samples which are leaking and/or not properly identified will be rejected. The following are brief guidelines for properly triple-packaging and shipping specimens for infectious testing at the Arizona State Public Health Laboratory:

- **Primary Container**
  - Must be securely sealed; leak-proof for liquids and sift-proof for solids
    - NOTE: screw caps and parafilm recommended
    - NOTE: Primary **OR** secondary container must be pressure and temperature capable (95kPa) if air transportation is used
  - Samples must be properly labeled with patient identifying information
    - Specimen primary containers for Mycobacterial examination must be labeled with the patients name, specimen type, date AND time of the collection.
  - 50mL or 50g max quantity
  - Wrap with absorbent material sufficient for entire contents, and cushioning material
- **Secondary Container**
  - Securely sealed and watertight/leak-proof
    - NOTE: Primary **OR** secondary container must be pressure and temperature capable (95kPa) if air transportation is used
    - NOTE: If you have the appropriate materials you can place multiple primary containers inside a secondary container
  - A completed itemized list of contents must be placed outside of, or surrounding the secondary container
    - NOTE: An ASPHL Submission Form will satisfy this requirement
  - Place absorbent and cushioning material between the primary and secondary containers
  - Affix a biohazard symbol to the secondary container

- **Tertiary/Outer Container for CATEGORY B shipments**
  - Outer package must be rigid and of good quality
  - Affix UN 3373 Biological Substance, Category B diamond shaped hazard label
    - Do not affix biohazard symbol to outer package
  - 4L or 4kg max quantity
  - Full name, complete address and phone number of shipper (responsible person)
  - Full name, complete address and phone number of recipient
- **Tertiary/Outer Container for CATEGORY A shipments**
  - Outer package and inner containers must be UN certified, outer package must contain the UN symbol
  - For Category A shipments containing infectious material affecting humans and animals; Affix UN 2814 Infectious Substances, Affecting Humans diamond shaped hazard label
  - For Category A shipments containing infectious material affecting animals only; Affix UN 2900 Infectious Substances, Affecting Animals diamond shaped hazard label
    - NOTE: The complete list of UN 2814 and UN 2900 organisms is contained at the end of this section
  - Cannot exceed 50mL or 50g if shipped in passenger aircraft
  - Cannot exceed 4L or 4kg if shipped in cargo aircraft
  - Orientation marks (up arrows) must be present on (2) sides of outer box
  - Full name, complete address and phone number of shipper (responsible person)
  - Full name, complete address and phone number of recipient
  - NOTE: Technical name of the organism may be omitted from the outer package, but must be present on the *Dangerous Goods Shipper's Declaration Form*
  - For Infectious Substance and Dry Ice Label template examples, refer to the ASPHL Shipping/Receiving website.
    - <http://www.azdhs.gov/lab/shipping-receiving.htm>
- **Additional Documentation and Considerations**
  - **Temperature Considerations**
    - Consult appropriate sections within this *Guide to Laboratory Services* document for specific shipping temperatures based on the organism or laboratory section performing test
    - If wet ice or ice packs are to be used for maintaining refrigerated shipping temperatures ensure there is sufficient absorbent material contained within to absorb all moisture if ice melts during transit so integrity of box is not compromised
      - NOTE: It is recommended to place wet ice and/or ice packs inside a zip-lock bag and surround this with absorbent material
    - If dry ice is to be used to maintain sub-frozen temperatures ensure that the package conforms to PI 954 and that dry ice is not placed inside any tightly sealed container that will prevent the release of carbon dioxide gas during sublimation.
      - NOTE: Dry ice will degrade rapidly therefore it must be purchased, obtained and used as close to actual shipping as possible.

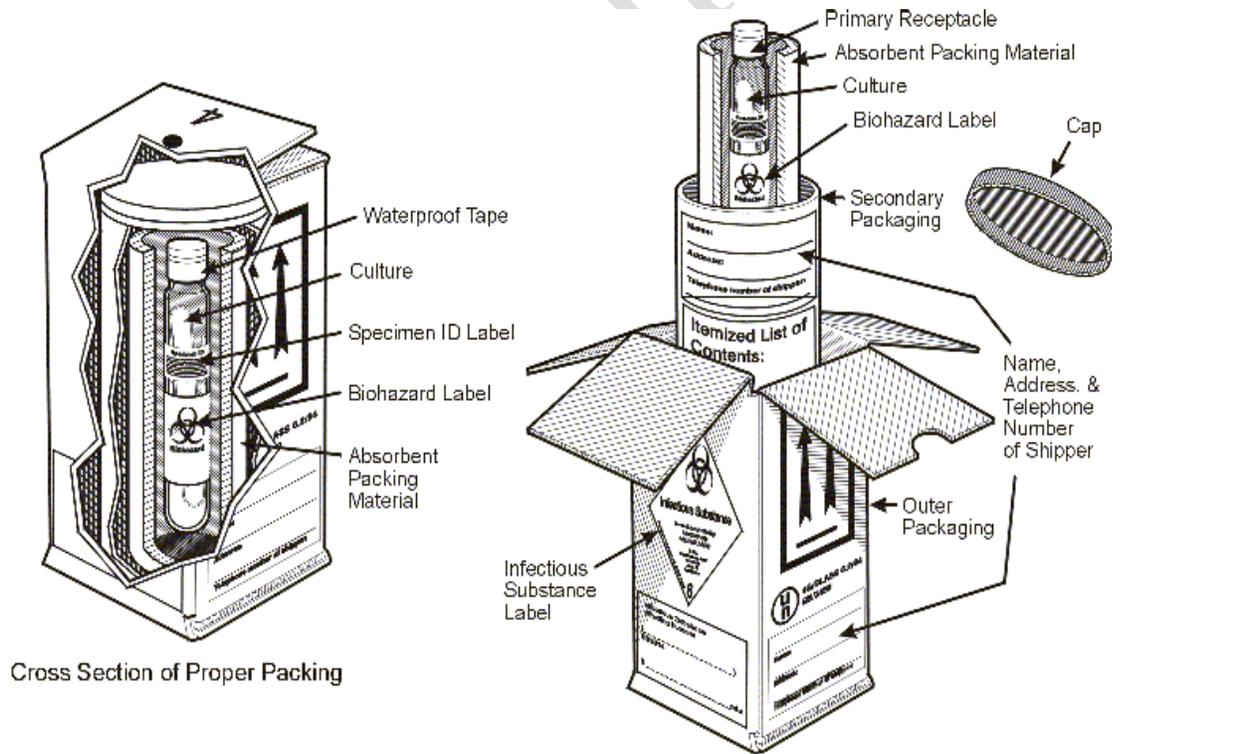
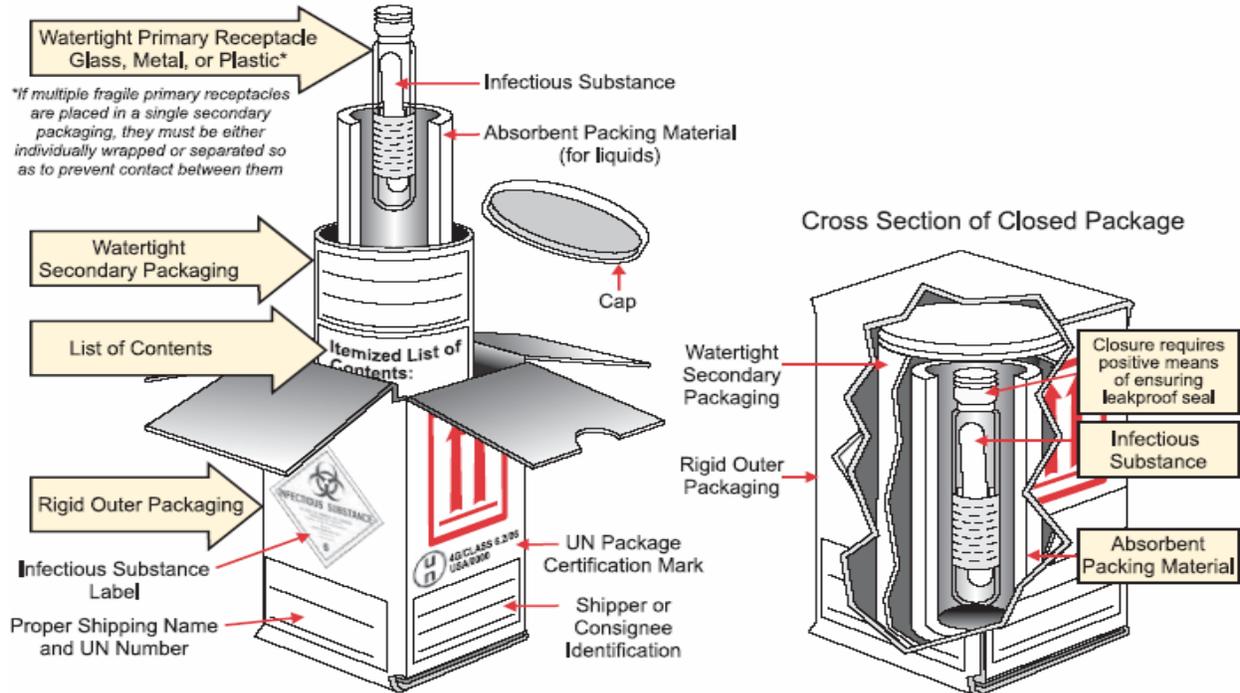
- **Dangerous Goods Shipper's Declaration**
  - A Shipper's Declaration must accompany all Category A shipments
    - NOTE: A minimum of 3 signed copies is needed
  - A Shipper's Declaration is not needed for Category B shipments
  - A Shipper's Declaration is not needed if only shipping dry ice, or dry ice with a Category B shipment
- **Select Agent and Toxin Transfers**
  - Shipping of any Select Agent or Toxin must have prior approval and a completed CDC/APHIS Form 2. For additional information please visit the Select Agents website at: <http://www.selectagents.gov/TransferForm.html> or contact the Arizona State Public Health Laboratory Bioemergency Response Section at 602-364-0999 for further assistance
- **Training**
  - Anyone who packages or ships infectious material must receive appropriate training. There are several commercial courses and trainings available. The Arizona State Public Health Laboratory offers a free course entitled "*Packaging and Shipping of Infectious Substances*" on a quarterly basis to train clinical laboratorians and healthcare professionals how to properly ship Category A and B samples to the Arizona State Public Health Laboratory. For further information or the next scheduled course please contact the ASPHL Bioemergency Response Laboratory at 602-364-0999
- **Supplies**
  - The Arizona State Public Health Laboratory offers several collection kits and materials for submitting samples. Please see Section 9 for further information
- **Regulations and Additional Guidance**
  - Arizona State Reporting and Isolate Submission Requirements A.A.C. R9-6-204 [www.azdhs.gov/phs/oids/reporting/labs.htm](http://www.azdhs.gov/phs/oids/reporting/labs.htm)
  - CDC/USDA Select Agent and Toxin list: <http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html>
  - American Society of Microbiology Sentinel Laboratory Shipping Guidance <http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf>
  - DOT (Department of Transportation) 49 CFR Part 171-180, Hazardous Materials Regulations: <http://hazmat.dot.gov> or <http://hazmat.dot.gov/regs/rules.htm>
  - United States Postal Service (USPS) 39 CFR Part 20, International Postal Service (International Mail Manual), and Part 111, General Information on Postal Service (Domestic Mail Manual). Regulations on transporting infectious substances through the USPS are codified in Section 601.10.17 of the Domestic Mail Manual and Section 135 of the International Mail Manual. A copy of the Domestic and International Mail Manuals may be obtained from the Government Printing Office by calling Monday through Friday, 7:30 a.m. - 9:00 p.m. EST: 202- 512-1800, 866-512-1800 (toll free), or from: <http://bookstore.gpo.gov/http://pe.usps.gov/>

- Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens: <http://www.osha.gov/>
- Technical Instructions for the Safe Transport of Dangerous Goods by Air (Technical Instructions). International Civil Aviation Organization (ICAO). A copy of these regulations may be obtained from the ICAO Document Sales Unit at (514) 954-8022, Fax: (514) 954-6769, E-Mail: [sales\\_unit@icao.int](mailto:sales_unit@icao.int), or from: <http://www.icao.int>
- Dangerous Goods Regulations International Air Transport Association (IATA). These regulations are issued by an airline association, are based on the ICAO Technical Instructions, and are followed by most airline carriers. A copy of these regulations can be obtained from: <http://www.iata.org/index.htm> or <http://www.who.int/en/>
- Please contact the Arizona State Public Health Laboratory (ASPHL) for appropriate specimen types and shipping instructions for specimen referral to the Center for Disease Control and Prevention (CDC) for non-routine testing not offered at the ASPHL.

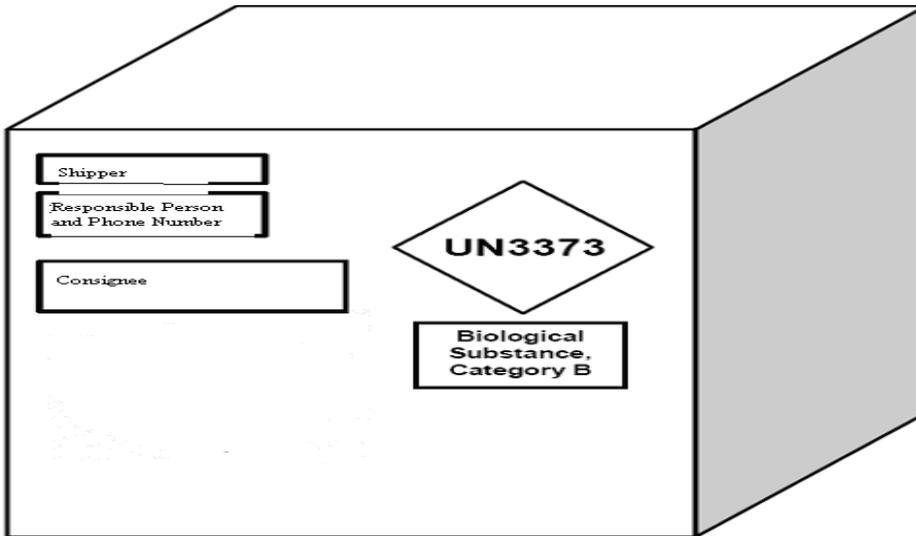
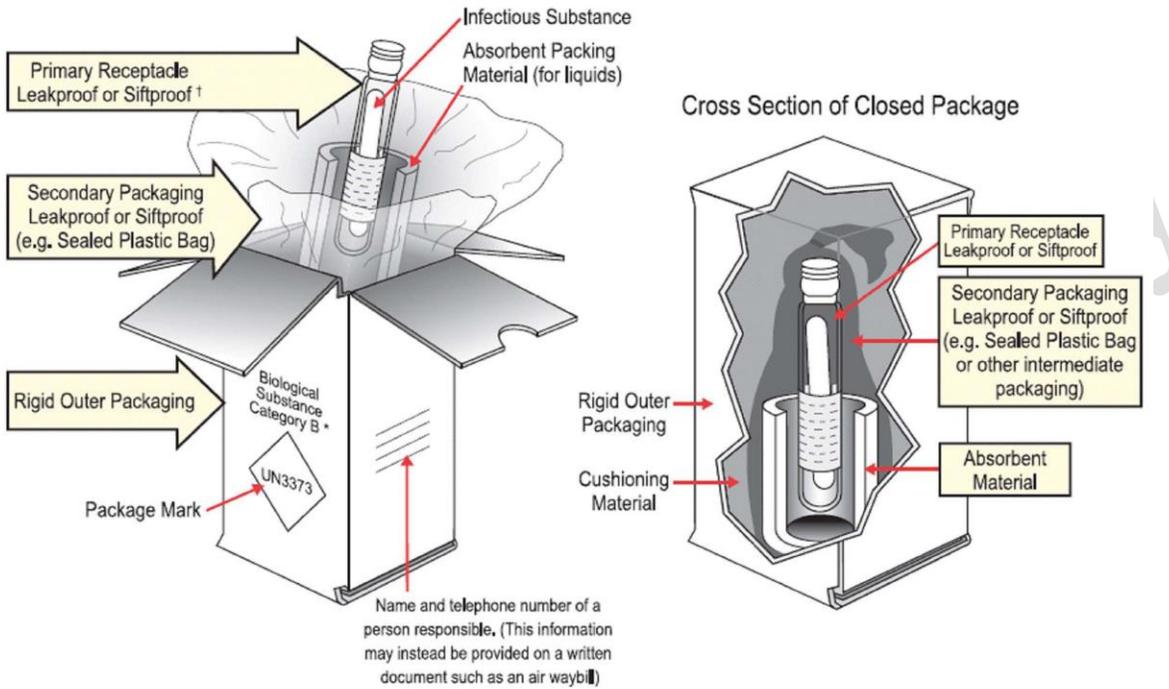
The following list is not exhaustive. It is the list of Category A organisms as outlined by the IATA regulations. If there is any doubt as to whether the shipment should be sent as Category A or B, please contact the Arizona State Public Health Laboratory at 602-542-1190 or 602-364-0999 for assistance.

Ref: IATA Dangerous Goods Regulation 52 <sup>nd</sup> Edition, January 2011: 3.6.2.2 Classification of Infectious Substances Table 3.6 D UN number and proper shipping name	Organism
UN 2814 Infectious substance, affecting humans	<i>Bacillus anthracis</i> (cultures only) <i>Brucella abortus</i> (cultures only) <i>Brucella melitensis</i> (cultures only) <i>Brucella suis</i> (cultures only) <i>Burkholderia mallei</i> (cultures only) <i>Burkholderia pseudomallei</i> (cultures only) <i>Chlamydia psittaci</i> (avian) (cultures only) <i>Clostridium botulinum</i> (cultures only) <i>Coccidioides immitis</i> (cultures only) <i>Coxiella burnetii</i> (cultures only) Crimean-congo hemorrhagic fever virus Dengue virus (cultures) Eastern equine encephalitis virus (cultures only) <i>Escherichia coli</i> , verotoxigenic (cultures only) Ebola virus <i>Francisella tularensis</i> (cultures only) Hantaan virus Hepatitis B virus (cultures only) Herpes B virus (cultures only) Human immunodeficiency virus (cultures only) Lassa virus Marburg virus <i>Mycobacterium tuberculosis</i> (cultures only) Poliovirus (cultures only) Rabies virus <i>Rickettsia prowazekii</i> (cultures only) <i>Shigella dysenteriae</i> type 1 (cultures only) Variola virus Venezuelan equine encephalitis virus West Nile virus (cultures only) Yellow fever virus (cultures only) <i>Yersinia pestis</i> (cultures only)
UN 2900 Infectious substance, affecting animals	Bluetongue virus Classical swine fever virus Foot-and-mouth disease virus Goatpox virus Lumpy skin disease virus Newcastle disease virus Sheeppox virus Swine vesicular disease virus Vesicular stomatitis virus

# Category A Shipping Examples



# Category B Shipping Examples



A completely labeled outer package. The primary container inside the package contains a Biological Substance, Category B substance and is packed according to PI 650.

Sample ID: \_\_\_\_\_  
Packager Name/Initial: \_\_\_\_\_  
Date: \_\_\_\_\_

## **CATEGORY B CHECKLIST**

-UN 3373 Biological Substances, Category B  
-IATA Packing Instruction (PI) 650  
-FedEx, UPS, USPS (Us Mail), private couriers

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### **Primary:**

- Specimen properly labeled with patient ID information
- 50mL or 50g maximum quantity
- Securely sealed & watertight/leakproof (screw cap receptacle and parafilm)  
Note: a Petri dish is not an acceptable primary container
- Wrapped in absorbent material sufficient for entire contents
- Wrapped in cushioning material (bubble wrap)
  - Primary **OR** secondary container pressure and temperature capable (95kPa)

### **Secondary:**

- Securely sealed and watertight/leakproof
  - Primary **OR** secondary container pressure and temperature capable (95kPa)
- A completed itemized list of contents (requisition or sample submission form) is placed between the secondary packaging and the outer packaging (NOT inside the secondary packaging)
- Absorbent material is placed between the primary and secondary packaging
- Biohazard symbol on secondary package required if shipping via US Mail (USPS)
- (Optional) Additional cushioning material placed between primary and secondary

### **Outer Package (Rigid):**

- Package is rigid and of good quality (acceptable to reuse Category B packages)
- UN 3373 Biological Substances, Category B diamond shaped label
- Quantity of infectious material is listed
- Quantity of sample –volume (mL) or weight (g)
- Must not contain more than 4 L, 4000mL or 4kg
- Do NOT put biohazard symbol on outer package
- Full name, complete address and phone number of person responsible for the shipment  
(This can be either the shipper or the recipient, but must be someone knowledgeable of the contents)
- Full name, complete address and telephone number of the shipper
- Full name, complete address and telephone number of the consignee/recipient

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NOTE: A Shipper's Declaration is not needed for Category B samples OR if dry ice is used.

If dry ice is used consult dry ice shipping checklist

If overpack is used consult overpack shipping checklist

Sample ID: \_\_\_\_\_  
Packager Name/Initial: \_\_\_\_\_  
Date: \_\_\_\_\_

### **DRY ICE CHECKLIST**

**Combined with Category A, B, or Exempt Shipments**  
**UN 1845 -Miscellaneous Hazard Class 9**  
**Packing Instruction (PI) 954 *\*formerly PI 904***

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- Properly ventilated package  
NOTE: Does not have to be a UN certified package
  - Overpack sticker/label needed for Category A shipments only  
(Category B packages will be packaged differently than A)
  - The net quantity of dry ice used in kg is listed
  - The quantity of the dry ice per package is less than 200kg
  - Irrelevant marks and labels removed from package
  - The UN number "UN 1845" label or sticker
  - Miscellaneous Hazard Class 9 label or sticker
  - Full name, complete address and phone number of person responsible for the shipment  
(This can be either the shipper or the recipient, but must be someone knowledgeable of the contents)
  - Full name, complete address and telephone number of the shipper
  - Full name, complete address and telephone number of the consignee/recipient
- 

### **Shipper's Declaration for Dry Ice**

Shippers Declaration must be completed for shipments containing Category A substances only, dry ice alone does not require a Shipper's Declaration

- The words "Carbon dioxide, solid" or "Dry ice" is contained on Shipper's Declaration  
Recommended to use "Dry Ice"
- Packing group III
- Packing Instruction 954

Consult Checklist for Shipper's Declaration for complete list

## Section 9: Requesting Collection Kits and Mailing Containers

Supplies ordered from the Arizona State Public Health Laboratory (ASPHL) are to be used ONLY to submit specimens to the ASPHL. There are two Requests for Materials forms currently in use: a *Newborn Screening Supplies Request Form* and a *Request Form* for all other supplies available from the ASPHL. Supplies can be requested by mailing, faxing, emailing or call the Receiving Section. All request forms are available as fillable or printable documents at <http://www.azdhs.gov/lab/shipping-receiving.htm>.

Arizona Department of Health Services  
Bureau of State Laboratory Services  
ATTN: Receiving Section  
250 North 17<sup>th</sup> Avenue  
Phoenix, AZ 85007  
Fax (602) 364-0758  
Phone (602) 542-1190  
Email [labreceiving@azdhs.gov](mailto:labreceiving@azdhs.gov)

Please request materials before they are required as the expected turn around time per order is **FIVE** business days. Most materials do have a limited shelf life; therefore, only order what will be used before the expiration date. Please do not use expired kits or any kits in which the medium has changed characteristics. Dispose of the media properly and order replacement supplies. The following table provides information regarding submission forms, kit contents and expiration period of each kit. Submitters may use the *Request for Materials Form* to order entire kits, as well as individual components.

KIT	CONTENTS	SHELF LIFE
Enteric Kit	Instruction Sheet Baggie Metal Container Cardboard Mailer Media: Cary Blair Store +20 to +25°C	1-2 years
Influenza Kit	The Microbiology Submission Form can be gotten from the website link below: <a href="http://www.azdhs.gov/lab/shipping-receiving.htm">http://www.azdhs.gov/lab/shipping-receiving.htm</a> Instruction Sheet N/P Swab Media: Universal Transport Medium Store +2 to +25°C	1-2 years
Leptospira Culture Media	Leptospira Media Instructions	6-12 months
Ova & Parasite Kit	<a href="http://www.azdhs.gov/lab/shipping-receiving.htm">http://www.azdhs.gov/lab/shipping-receiving.htm</a> Instruction Sheet Metal Container Cardboard Mailer 10% Formalin. Store +20 to +25°C	1-2 years
Pertussis Kit	The Microbiology Submission Form can be gotten from the website link below: <a href="http://www.azdhs.gov/lab/shipping-receiving.htm">http://www.azdhs.gov/lab/shipping-receiving.htm</a> Instruction Sheet N/P Swab Media: Regan Lowe. Store +2 to +8°C	4-6 months
Tuberculosis Kit	<a href="http://www.azdhs.gov/lab/shipping-receiving.htm">http://www.azdhs.gov/lab/shipping-receiving.htm</a> Sputum Vial Metal Container Cardboard Mailer Store +20 to +25°C	