

**CLINICAL SPECIMENS  
COLLECTION AND PACKAGING  
FOR SELECT AGENT TESTING**

*NEW YORK STATE DEPARTMENT OF HEALTH*

*Wadsworth Center*

*Bacteriology Laboratory*

*120 New Scotland Avenue*

*Albany, NY 12208*

**General Instructions:**

The diagnostic facilities of the Wadsworth Center are designed primarily to give assistance to health officers and physicians in diagnosis and control of communicable diseases, and to provide laboratory service for persons in the state for whom service is not otherwise available. The Wadsworth Center is also a designated Level 3 laboratory under the national laboratory defense network and will conduct definitive diagnostic work on biological or chemical specimens in a suspected terrorist event.

Always contact the Wadsworth Center Laboratories before submitting a specimen. Special sample transportation arrangements may be necessary.

For a suspected act of terrorism:

Call 518-474-4177 between 8:00 AM to 5:00 PM work days or  
Call the Duty Officer 866-881-2809 after hours and weekends/holidays.

**Biohazard Specimens are considered legal evidence relating to non-accidental exposure to nuclear, biological, and chemical (NBC) agents. The Wadsworth Center will accept Departmental approved specimens (physical evidence) from members of the health services system or law enforcement agencies. Material submitted as physical evidence must comply with the policies set forth to ensure appropriate chain of custody and specimen handling guidelines as described below.**

**Protocols for Collection and Packaging of Clinical Specimens**

**Collection**

**History forms:** All samples must include a history form.

1. Carefully complete the history form, including clinical information to insure optimal processing of the specimen.
2. Submit pure cultures of bacteria growing on solid media in screw-capped tubes or vials (for anaerobes, in chopped meat), or in suitable transport media. DO NOT send plates.

3. Submit clinical specimens in suitable leak-proof tubes, jars, or containers. Tighten the cap of the container to prevent leakage.
4. Label with the patient name.

Packaging: Refer to packaging instructions.

**Transportation:** Specimens can be mailed, sent by express carrier or by courier to the laboratory. Special arrangement for same day delivery to the lab can be made. Some clinical specimens require special shipping conditions –e.g. specimens for *Clostridium botulinum* toxin testing and other bacterial toxins. Please contact the Laboratories at 518-474-4177 before submitting a specimen.

### **Bacillus anthracis (anthrax)**

#### **COLLECTION: Cutaneous Anthrax**

Vesicular stage:

1. The organism is best demonstrated in this stage.
2. Collect vesicular exudate found in early lesions of cutaneous anthrax by soaking 2 swabs with vesicular fluid from a previously unopened vesicle.

Eschar stage:

1. In well-formed eschar, in which vesicular exudate is absent, the edge of the eschar should be lifted up with sterile forceps without removing the eschar.
2. Fluid should be obtained by application of a sterile capillary tube under the edge or rotation of two swabs beneath the edge of the eschar without removing the eschar.

#### **Pulmonary Anthrax**

If respiratory symptoms are present and sputum is being produced, obtain a sputum specimen for smear and culture.

For the severely ill patient, blood cultures should be obtained. Use routine blood culture methods. Blood cultures may yield the organism, especially if specimens are drawn before antibiotic treatment.

Postmortem blood collected by venipuncture (a characteristic of anthrax is nonclotting blood at death) should be examined by culture and M'Fadyean-stained smear. Any hemorrhagic fluid, from nose, mouth, or anus should be cultured. If these cultures are positive, no further specimens are needed. If they are negative, specimens of peritoneal fluid, spleen, and/or mesenteric lymph nodes, aspirated by techniques that avoid spillage of fluids, may be collected for smear and culture.

#### **Gastrointestinal Anthrax**

If the intestinal anthrax patient is not severely ill and able to produce a stool specimen, a fecal specimen should be collected.

If the patient is severely ill, blood cultures should be collected. Blood cultures may yield the organism, especially if specimens are drawn before antibiotic treatment.

Postmortem, the approach given for pulmonary anthrax should be followed.

### **Other Specimens**

Isolates of large, gram positive, endospore-forming bacilli suspected to be *Bacillus anthracis* should be sent to a reference laboratory for identification confirmation.

### **Yersinia pestis (plague)**

#### **COLLECTION:**

#### **Bubonic plague**

Lymph node aspirate is the specimen of choice. It may be necessary to inject a small amount of sterile saline into a node before aspirating material, because nodes in plague-infected patients are not usually purulent. Excised tissue specimens may be submitted in a sterile container with sterile saline. Blood cultures should be taken whenever possible.

#### **Septicemic plague**

Collect a minimum of 20ml of blood in a blood collection tube with SPS (sodium polyanethole sulfonate). *Yersinia pestis* may grow in routine blood culture media. Bacteria may be intermittently released from affected lymph nodes into the bloodstream: therefore, a series of blood specimens taken 10-30 minutes apart may be productive in the isolation of *Y. pestis*. These organisms are fastidious in their growth requirements and often require prolonged incubation time for detection.

#### **Pneumonic plague**

Bronchial/tracheal washing should be taken from suspected pneumonic plague patients; sputum or throat swab may be collected, but are not ideal for isolation of plague since they often contain many other bacteria that can mask the presence of plague.

#### **Other Specimens**

*Y. pestis* may be present in cerebrospinal fluid, feces, and urine, depending on the clinical form and stage of the disease.

Postmortem specimen of lymphoid tissues, lung and bone marrow samples may yield evidence of *Y. pestis*.

Isolated of gram-negative bacilli, suspected to be *Y. pestis* should be sent to a reference laboratory for identification confirmation.

**Referral for *Y. pestis* testing:** If referring a clinical primary specimen – Ship with refrigerants.  
If referring culture – No refrigerants required.

### ***Clostridium botulinum* toxin and culture (botulism)**

#### **COLLECTION:**

##### **Infant botulism**

Feces-collect as much stool as possible; a minimum of 10 grams is preferred.

Serum-is NOT recommended for infant botulism testing.

##### **Adult and older children botulism**

Serum and feces are the preferred specimens for examination. Gastric contents and intestinal contents may also be examined.

Serum-a minimum of 5 ml of serum (not whole blood) is required;  
10 ml or more is preferred, as it will permit typing of the toxin.

Feces and Gastric contents-a minimum of 10 grams is required for toxin analysis. The submitter should send as much fecal/gastric contents specimen as possible. If the patient has been taking any medication that might interfere with toxin assays or culturing the stool, the laboratory should be notified.

##### **Suspect Food/Water**

**Food-** a minimum of 10 grams is required; it is preferred to collect as large a specimen as possible. Empty containers with remnants of suspected foods can be examined. Foods should be left in the original containers if possible or placed in sterile unbreakable containers and labeled carefully. Place containers individually in leak proof containers (i.e. sealed plastic bags) to prevent cross-contamination during shipment.

**Water-** a minimum of 100 ml is required. Collect water in a sterile unbreakable container and label carefully. Place container (s) individually in leak proof containers (i.e. sealed plastic bags) to prevent cross-contamination during shipment.

##### **Wound botulism**

Specimens include serum or samples from the wound, including tissue, exudates or swabs.

##### **Botulism Specimen Transport:**

1. Please notify the NYS Clinical Bacteriology Laboratories at 518-474-4177 before transport of specimen.
2. Specimens should be sent refrigerated as rapidly as possible! Overnight courier or special messenger is required. Specimens should be placed in sterile leakproof containers, then in insulated shipping containers with freezer packs. DO NOT freeze specimen.

3. The following specimens are NOT tested on a STAT basis: Infant botulism, and adult botulism with symptoms greater than 5 days. Store non-stat specimens collected during weekend at refrigerator temperature of 4C.

**Criteria for Specimen Rejection:**

Specimens that have not been refrigerated after collection and during transport to the lab, or sent in a timely manner may be rejected.

**Francisella tularensis (tularemia)**

**COLLECTION:**

Tularemia is an illness characterized by several distinct forms: ulceroglandular, glandular, olcuglandular, oropharyngeal, intestinal, pneumonic, and typhoidal.

**Specimens of choice:**

1. Lymph node aspirate
2. Ulcer material/scraping, tissue (biopsy sample from advancing edge of the lesion, not central necrotic area which is usually secondarily infected).
3. Sputum, throat swab, bronchial washing
4. Gastric washings
5. Blood Cultures

Note: If a specimen needs to be shipped to the laboratory for processing, it should be kept cold but not frozen. No transport medium is necessary.

**Other Specimens:**

1. Isolates of gram-negative coccobacilli, suspected to be *Francisella tularensis* should be sent to a reference laboratory for identification confirmation.
2. Paired serum specimens (acute/convalescent) for serology tests (detection of antibody to *F. tularensis*). The acute-phase serum should be collected as close to the onset of illness as possible and convalescent-phase serum should be collected 21 days later. Please contact NYS Clinical Bacteriology Laboratories at 518-474-4177 before transport of serum specimens.

**Brucella species (brucellosis)**

**COLLECTION:**

**Blood**

A minimum of 20ml of blood in a blood collection tube with SPS (sodium polyanetholesulfonate). *Brucella* spp. will grow in routine blood culture media. Multiple specimens enhance the probability of obtaining a positive culture; a series of blood

specimens taken 10-30 minutes apart may be productive in the isolation of *Brucella* spp.

### **Bone Marrow Aspirate**

Draw 0.5 to 1.0 ml from the iliac crest.

1. Directly inoculate and streak for isolation the following plating media (within 2 hours of collection): 5% sheep blood agar, horse blood agar, chocolate agar, Martin-Lewis or Modified Thayer-Martin agar. Incubate at 35-37C in 5-10% CO<sub>2</sub>. If the aspirate specimen needs to be shipped to the laboratory for processing, it should be kept cold but not frozen.

2. Or inoculate blood culture bottle(s) with the bone marrow aspirate.

### **Other Specimens:**

1. Other specimens from which *Brucella* spp. have been recovered are lymph node aspirate, synovial fluid, CSF, abscess aspirates, and liver and spleen biopsy specimens. Blood culture systems may be used for other fluids.

2. Isolates of gram-negative bacilli, suspected to be *Brucella* spp. should be sent to a reference laboratory for identification confirmation.

3. Paired serum specimens (acute/convalescent) for serology tests (detection of antibody to *Brucella* spp.). The acute-phase serum should be collected as close to the onset of illness as possible and convalescent-phase serum should be collected 21 days later. Specimens should be shipped and stored frozen. Please contact NYS Clinical Bacteriology Laboratories at 518-474-4177 before transport of serum specimens.

## **Packaging Instructions**

### **Regulations**

Public Health Service 42 CFR Part 72. Interstate Transportation of Etiologic Agents. This regulation is in revision to harmonize it with the other U.S. and international regulations. A copy of the current regulation may be obtained from the Internet at: <http://www.cdc.gov/od/ohs>

Department of Transportation (DOT). 49 CFR Parts 171-178. Hazardous Materials Regulations. Applies to the shipment of both biological agents and clinical specimens. Information may be obtained from the Internet at: <http://www.dot.gov.rules.html>

United States Postal Service. 39 CFR Part 111. Ability to Mail Etiologic Agents. Codified in the Domestic Mail Manual 124.38: Etiologic Agent Preparations. A copy of the Domestic Mail Manual may be obtained from the Government Printing Office by calling 1-202-512-1800 or from the Internet at: <http://www.access.gpo.gov>

**General Packaging Requirements for Transport of Biological Agents:**

All specimens and isolates are to be placed in approved mailing containers, shipped, and labeled in accordance with DOT Federal Regulation 42 CFR Part 72 and U.S. Postal Regulation 39 CFR Part iii. **At a minimum, International Air Transport Authority (IATA) and DOT regulations must be followed.**

If a biothreat agent has been suspected within a clinical specimen, *over-night delivery* to the Wadsworth Center is recommended, and therefore, must be packaged and shipped according to appropriate IATA requirements. According to IATA regulations, clinical specimens should be shipped as **Biological substances, category B** using the proper shipping label **UN3373**.

For more information pertaining to IATA regulations, visit: <http://www.iata.org>