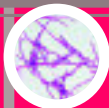


2004 Bioterrorism Agents & Diseases

State of Florida Lab Reference Guide



Bacillus anthracis (Anthrax) p. 1
BSL-2 AGENT



Yersinia pestis (Plague) p. 4
BSL-2 AGENT



Clostridium botulinum (Botulism) p. 7
BSL-2 AGENT



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BSL-3 AGENT



Variola major (Smallpox) p. 14
BSL-4 AGENT



Viral Hemorrhagic Fever p. 18
BSL-4 AGENT

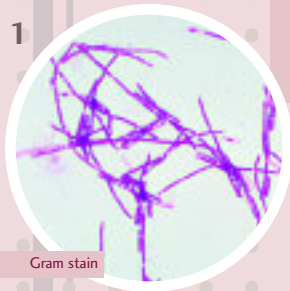
HOT LINE PHONE NUMBERS

- Florida Department of Health,
Bureau of Epidemiology
(850) 245-4401 24/7
- Florida Department of Health,
Bureau of Laboratories,
State Laboratory Numbers
Jacksonville
(904) 591-7406 24/7, cell
Miami
(305) 366-0025 24/7, pager
Tampa
(813) 883-5929 24/7, pager

• See page 10 for county health department numbers.

- For shipping questions contact the Florida Department of Transportation
1-800-467-4922

Sample labeling information; Paperwork requirements for samples; Rejection criteria; Special specimen collection notation; Abbreviations; Definitions
p.21



Gram stain

Bacillus anthracis (Anthrax)

BSL-2 agent Procedures should be performed in a lab that utilizes Biological Safety Level 2 (BSL-2) practices and a biological safety cabinet (BSC) is highly recommended.

Good hand washing is essential.

SPECIMEN COLLECTION

Inhalational

BRONCHOALVEOLAR LAVAGE Collect > 1 ml into a sterile container.

Inhalation anthrax does not result in sputum formation.

BLOOD CULTURES † Collect appropriate blood volume and number of sets per lab protocol.

Gastrointestinal

BLOOD CULTURES † Collect appropriate blood volume and number of sets per lab protocol.

In later stages of disease (2–8 days post-exposure) blood cultures may yield the organism, especially if obtained before antibiotic treatment.

STOOL Transfer ≥5 grams of stool into a clean, dry, sterile, wide mouth, leak proof container.

RECTAL SWAB † For patients unable to pass a specimen, obtain a rectal swab by carefully inserting a swab 1 inch beyond the anal sphincter.

Cutaneous

VESICULAR STAGE † Aseptically collect vesicular fluid on sterile swabs from previously unopened vesicles.

Anthrax bacilli are most likely to be seen on Gram stain at this stage.

ESCHAR STAGE † Collect eschar material by carefully lifting the eschar's outer edge; insert a sterile swab, then slowly rotate for 2–3 seconds beneath the edge of the eschar without removing it (use of a smaller swab such as urethral culture swab or nasopharyngeal type may be helpful to accomplish this).

NASAL SPECIMENS FOR SCREENING † Use a moistened (saline or sterile H₂O) synthetic swab taken at least 1 cm inside the nares. Rotate the swab and leave it in place for 10–15 seconds. Withdraw and insert into its transport container.

Should only be used to support a confirmed exposure to B. anthracis or during an ongoing epidemiologic investigation. Gram stain for spores is not recommended.

† Before specimen collection, please refer to pg. 21.

Bacillus anthracis (Anthrax)

TRANSPORT/STORAGE OF SPECIMENS TO LABORATORY

SWABS Transport in a sterile tube directly to laboratory at room temperature (RT). For transport > 1 hour, transport at 2–8°C.

Exception: do not refrigerate nasal screening swabs for culture.

STOOL Transport unpreserved stool to laboratory within 1 hour. For transport time > 1 hour, transport at 2–8°C; Cary-Blair or equivalent transport media is acceptable.

SPUTUM Transport in sterile, screw-capped container at RT when transport time in < 1 hour. For transport time > 1 hour, transport at 2–8°C.

BLOOD CULTURE Transport directly to laboratory at RT.

SENTINEL (LEVEL A) LABORATORY TESTS

GRAM STAIN large Gram positive rod (1–1.5 X 3–5µm)

CAPSULE DEMONSTRATION by India Ink procedure on smears prepared directly from clinical specimens

GROWTH CHARACTERISTICS ON AGAR (ground glass appearance, sticky-like beaten egg whites), non-hemolytic colonies

SPORULATION IN AIR (spores are oval located central-subterminally and are 1 X 1.5µm)

LACK OF MOTILITY

PENICILLIN ZONE INHIBITION (15–20 mm)

CATALASE POSITIVE

REFER TO REFERENCE LABORATORY

CONFIRMATION TESTING (Lysis by Gamma Phage, DFA, PCR, TRF)

Culture, PCR and Stains

ISOLATE (plate or slant) at RT

SWABS † (nasal passage lesion) moistened swab in tube with sterile saline or sterile water at RT

BLOOD (whole in EDTA or Citrate tubes) >1 ml, chilled or at RT

FLUIDS/ASPIRATE (pleural, bronchial) 0.5 ml, chilled

TISSUE (fresh) 5mm³ in container, frozen

BLOOD CLOT 1.0 ml clot, chilled or frozen

SERUM 1.0 ml, chilled (NOT frozen)

PLASMA 1.0 ml, chilled (NOT frozen)

Serology

SERUM (separated) >1 ml, chilled (NOT frozen)

CITRATED PLASMA (separated) >1 ml, chilled (NOT frozen)

† Before specimen collection, please refer to pg. 21.

Bacillus anthracis (Anthrax)

TRF

ISOLATE (plate or slant) at RT
SERUM >1 ml, chilled
NASOPHARYNGEAL SWAB † at RT
BRONCHIAL WASHING/TRACHEAL ASPIRATE, chilled
LESION EXUDATES, chilled

Histopathology

TISSUES preserved, 1.0cm³, at RT
BIOPSIES OF LESIONS, E.G. SKIN Preserved, 0.3mm diameter, at RT.

SHIPPING REQUIREMENTS: SPECIMENS SENT FROM SENTINEL LAB TO REFERENCE LAB

Contact your DOH state laboratory director if you represent a laboratory that would like to ship a biologic agent to an approved advanced capacity laboratory for presumptive or confirmatory identification. Inform the DOH State Laboratory Director as to the identity of the suspected critical biologic agent.

Follow guidelines for packing and labeling using IATA Packaging Instruction 602 for infectious substances or 650 for diagnostic specimens or DOT 49 CFR 173.196 for infectious substances or 49 CFR 173.199 for diagnostic specimens (using the more restrictive regulation). General packaging requirements for transport of biological agents includes triple (primary receptacle, water tight secondary packaging, durable outer packaging) packaging and requires the “Infectious Substance” label on the outside of the package.

Shippers declaration of dangerous goods must be completed
If the reference lab confirms the presence of a Select Agent then all samples, clinical specimens, and isolates stored at the sentinel lab (your facility) must be transferred to the approved reference lab immediately. In addition, CDC form 0.1318 must be filled out by both the sentinel (your facility) and reference lab and sent to CDC within 7 days. ●

† Before specimen collection, please refer to pg. 21.

Yersinia pestis (Plague)

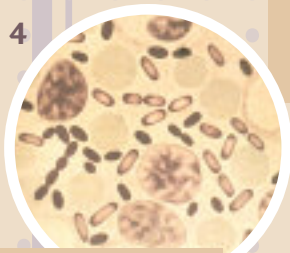


Photo micrograph showing bipolar staining

BSL-2 agent Procedures should be performed in a lab that utilizes Biological Safety Level 2 (BSL-2) practices and a biological safety cabinet (BSC) is highly recommended.

SPECIMEN COLLECTION

Pneumonic

BRONCHIAL WASHING OR TRANSTRACHEAL ASPIRATE (>1 ML)

Sputum may be examined but this is not advised because of contamination by normal throat flora.

SEPTICEMIA † Blood cultures-Collect appropriate blood volume and number of sets per established laboratory protocol.

Organism is transient; may be necessary to collect sets 10–30 minutes apart.

Bubonic

ASPIRATE OF INVOLVED TISSUE (BUBO) OR BIOPSIED SPECIMEN

Liver, spleen, bone marrow and lung. Submit tissue or aspirate (syringe and needle can be sent if capped and secured with tape) in a sterile container. For small samples, add 1–2 drops of sterile normal saline to keep tissue moist.

Aspirates may yield little material; a sterile saline flush may be needed to obtain an adequate amount of specimen.

TRANSPORT/STORAGE OF SPECIMENS TO LABORATORY

RESPIRATORY CULTURES Transport in sterile, screw-capped containers at RT. For transport > 1 hour, transport at 2–8°C.

BLOOD CULTURES Transport samples directly to the laboratory at ambient temperature until they can be placed into the blood culture incubator. DO NOT refrigerate. Follow established lab protocols for processing blood cultures.

TISSUE ASPIRATE/BIOPSY Transport at RT for immediate processing or chilled if processing will be delayed.

SWABS † A swab of tissue is NOT recommended. However, if sent, the swab should be reinserted into the package for transport.

† Before specimen collection, please refer to pg. 21.

SENTINEL (LEVEL A) LAB TESTS**GRAM STAIN** Plump Gram negative rods (1–2µm X 0.5µm)**NON-LACTOSE FERMENTER****OXIDASE NEGATIVE****CATALASE POSITIVE****UREA NEGATIVE****INDOLE NEGATIVE****ROUTINE CULTURE MEDIA** (Cultures will grow slowly at 35–37°C and optimal at 28°C.)**CHARACTERISTICS ON AGAR AT 48 HOURS** gives the appearance of a “fried egg” or “hammered copper,” shiny surface on SBA.**GROWTH CHARACTERISTICS IN BROTH** (general nutrient); grow in clumps as “flocculant” or “stalactite” in appearance. Two tubes should be inoculated; then one incubated at 22–28°C and the other at 35–37°C; **DO NOT** shake or rock the tubes.*Additional smears using Wright-Giemsa or Wayson stains are reliable for accurately highlighting the bipolar staining characteristics on smears prepared directly from clinical specimens.***REFER TO REFERENCE LABORATORY****CONFIRMATION TESTING** (By Bacteriophage Lysis, DFA, Antibody Serology, PCR and TRF)**Culture and PCR****ISOLATE** (plate or slant) at RT**NASOPHARYNGEAL SWAB** † PCR no transport medium, dry ice or chilled. Culture-transport medium, chilled**SPUTUM**, chilled**BRONCHIAL WASHING/TRANSTRACHEAL ASPIRATE**, chilled**BLOOD** (whole in EDTA or Citrate tubes) 1 ml, chilled or at RT**Stains****ISOLATE** (plate or slant) at RT**PNEUMONIC-BRONCHIAL WASHING/TRANSTRACHEAL ASP., AND SPUTUM** <1 ml, chilled**BUBONIC-ASPIRATE OF INVOLVED TISSUE OR BIOPSIED SPECIMEN**

Submit tissue or aspirate (syringe and needle can be sent if capped and secured with tape) in a sterile container. For small samples, add 1–2 drops of sterile normal saline to keep tissue moist. Transport at RT for immediate processing or chilled if processing will be delayed.

† *Before specimen collection, please refer to pg. 21.*

Serology

SERUM (separated) >1 ml, chilled (NOT frozen)
CITRATED PLASMA (separated) >1 ml, chilled (NOT frozen)

TRF

ISOLATE (plate or slant) at RT
LYMPH NODE ASPIRATE, chilled
SERUM, 0.5ml chilled
NASOPHARYNGEAL SWAB †
BRONCHIAL WASHING/TRANSTRACHEAL ASPIRATE, chilled
LESION EXUDATES, chilled

Autopsy/biopsy specimen

Abscess material, lymph node, lung, liver, spleen, CSF and bone marrow scrapings. Transport at RT for immediate processing or chilled if processing will be delayed.

SHIPPING REQUIREMENTS: SPECIMENS SENT FROM SENTINEL LAB TO REFERENCE LAB

Contact your DOH state laboratory director if you represent a laboratory that would like to ship a biologic agent to an approved advanced capacity laboratory for presumptive or confirmatory identification. Inform the DOH State Laboratory Director as to the identity of the suspected critical biologic agent.

Follow guidelines for packing and labeling using IATA Packaging Instruction 602 for infectious substances or 650 for diagnostic specimens or DOT 49 CFR 173.196 for infectious substances or 49 CFR 173.199 for diagnostic specimens (using the more restrictive regulation). General packaging requirements for transport of biological agents includes triple (primary receptacle, water tight secondary packaging, durable outer packaging) packaging and requires the “Infectious Substance” label on the outside of the package.

Shippers declaration of dangerous goods must be completed
If the reference lab confirms the presence of a Select Agent then all samples, clinical specimens, and isolates stored at the sentinel lab (your facility) must be transferred to the approved reference lab immediately. In addition, CDC form 0.1318 must be filled out by both the sentinel (your facility) and reference lab and sent to CDC within 7 days. ●

† Before specimen collection, please refer to pg. 21.

Clostridium botulinum (Botulism)

BSL-2 agent Botulinum toxins are extremely poisonous. All materials suspected of containing toxin must be handled with **CAUTION!**



Gentian violet stain

SPECIMEN COLLECTION

Clinical samples

FECES Place into sterile unbreakable container and label carefully.

Confirmatory evidence of botulism may be obtained from 10–50 gram quantities (walnut size) or “pea-sized” stool samples in infants.

Anticholinesterase given orally, as in patients with Myasthenia gravis, has been shown to interfere with toxin testing.

ENEMA Place ~20 ml into sterile unbreakable container and label carefully. If an enema must be given because of constipation, a

minimal amount of fluid (preferably sterile, nonbacteriostatic water) should be used to obtain the specimen so that the toxin will not be unnecessarily diluted.

GASTRIC ASPIRATE OR VOMITUS Place ~20 ml into sterile unbreakable container and label carefully.

NASAL-ANAEROBIC SWABS † May be obtained for exposure to aerosolized botulinum toxin.

SERUM Use red top or separator tubes to obtain serum (no anticoagulant). Samples should be obtained as soon as possible after the onset of symptoms and before antitoxin is given. Enough blood should be collected to provide at least 10 ml of serum for mouse toxicity tests (usually 20 ml whole blood); serum volumes less than 3 ml will provide inconclusive results. Whole blood should NOT be sent as it typically undergoes excessive hemolysis during transit.

TISSUES, WOUND SWABS OR EXUDATES † Place into sterile unbreakable container and label appropriately. Alternatively, specimens can also be placed in Port-A-Cult vials* and sent to the appropriate lab, without refrigeration.

† Before specimen collection, please refer to pg. 21.

* Becton Dickinson; catalog #4321609 or equivalent.

Clostridium botulinum (Botulism)

POSTMORTEM Obtain specimens of intestinal contents from different levels of small and large intestines. Place ~10 grams per specimen into sterile unbreakable container and label carefully. Obtain gastric content, serum, and tissue specimens if/as appropriate (see page 9 for appropriate collection).

TRANSPORT/STORAGE OF SPECIMENS TO LABORATORY

FECES Transport directly to the laboratory at 4°C. (*see note below**)

ENEMA Transport directly to the laboratory at 4°C.

GASTRIC ASPIRATE OR VOMITUS Transport to the laboratory at 4°C.

NASAL SWABS Transport to laboratory directly at RT in an anaerobic transport medium.

SERUM Transport directly to the laboratory at 4°C. (*see note below**)

TISSUE OR EXUDATES Transport anaerobic swabs or transport system at RT.

POSTMORTEM/INTESTINAL FLUID Transport directly to laboratory at 4°C.

**If an unavoidable delay of several days is anticipated, the specimens (serum or stool) should be kept frozen and then packed in an insulated container with dry ice. Freezing does not significantly affect the stability of botulinum toxin in specimens; freezing does reduce the probability of recovering C. botulinum. Since direct detection of toxin provides the best laboratory confirmation of botulism, priority should be given to preserving preformed toxin prior to transport.*

SENTINEL (LEVEL A) LABORATORY TESTS

Level A laboratory procedures are designed to ensure the proper collection and distribution of appropriate specimens to designated testing laboratories.

REFER TO REFERENCE LABORATORY

CONFIRMATION TESTING (Mouse bioassay and isolate testing)

Clinical Samples

FECES, chilled

ENEMA, chilled

GASTRIC ASPIRATE OR VOMITUS, chilled

NASAL SWABS † at RT

SERUM, chilled

TISSUE OR EXUDATES, PORT-A-CULT VIALS at RT

POSTMORTEM, chilled

† *Before specimen collection, please refer to pg. 21.*

Clostridium botulinum (Botulism)

CULTURE Ship suspicious isolates anaerobically (overlay liquid media with 2-inch sterile petroleum jelly or petrolatum; melt/temper prior to overlaying culture). Cultures may be shipped at RT or chilled.

FOOD SPECIMENS Foods should be left in their original containers if possible, or placed in sterile unbreakable containers and labeled carefully. Place containers individually in leakproof containers (i.e. sealed plastic bags) to prevent cross contamination during shipment. Empty containers with remnants of suspected food can be examined, chilled.

SWAB SAMPLE Send clinical swabs † in an anaerobic transport medium (e.g. Port-A-Cult tubes) at RT or chilled.

SHIPPING REQUIREMENTS: SPECIMENS SENT FROM SENTINEL LAB TO REFERENCE LAB

Contact your DOH state laboratory director if you represent a laboratory that would like to ship a biologic agent to an approved advanced capacity laboratory for presumptive or confirmatory identification. Inform the DOH State Laboratory Director as to the identity of the suspected critical biologic agent.

Follow guidelines for packing and labeling using IATA Packaging Instruction 602 for infectious substances or 650 for diagnostic specimens or DOT 49 CFR 173.196 for infectious substances or 49 CFR 173.199 for diagnostic specimens (using the more restrictive regulation). General packaging requirements for transport of biological agents includes triple (primary receptacle, water tight secondary packaging, durable outer packaging) packaging and requires the “Infectious Substance” label on the outside of the package.

Shippers declaration of dangerous goods must be completed If the reference lab confirms the presence of a **Select Agent** then all samples, clinical specimens, and isolates stored at the sentinel lab (your facility) must be transferred to the approved reference lab immediately. In addition, CDC form 0.1318 must be filled out by both the sentinel (your facility) and reference lab and sent to CDC within 7 days. ●

† Before specimen collection, please refer to pg. 21.

Alachua (352) 334-7900

Baker (904) 259-6291

Bay (850) 872-4455

Bradford (904) 964-7732

Brevard (321) 634-6336

Broward (954) 467-4700

Calhoun (850) 674-5645

Charlotte (941) 639-1181

Citrus (352) 527-0068

Clay (904) 269-6340

Collier (239) 732-2684

Columbia (386) 758-1037

Dade (305) 376-1976

Desoto (863) 993-4601

Dixie (352) 498-1360

Duval (904) 665-2699

Escambia (850) 595-6500

Flagler (386) 437-7350

Franklin (850) 653-2111

Gadsden (850) 875-7200

Gilchrist (352) 463-3120

Glades (863) 946-0707

Gulf (850) 227-1276

Hamilton (386) 792-1414

Hardee (863) 733-4161

Hendry (863) 674-4141

Hernando (352) 754-4067

Highlands (863) 386-6040

Hillsborough (813) 307-8000

Holmes (850) 547-3691

Indian River (772) 794-7478

Jackson (850) 526-2412

Jefferson (850) 342-0170

Lafayette (386) 326-2708

Lake (352) 589-6424

Lee (239) 332-9577

Leon (850) 487-3186

Levy (352) 486-5300

Liberty (850) 643-2415

Madison (850) 973-5000

Manatee (941) 748-0747

Marion (352) 629-0137

Martin (772) 221-4030

Monroe (305) 292-6894

Nassau (904) 277-7287

Okaloosa (850) 833-9240

Okeechobee (863) 462-5760

Orange (407) 836-2502

Osceola (407) 343-2016

Palm Beach (561) 840-4568

Pasco (727) 869-3900

Pinellas (727) 824-6900

Polk (863) 519-7900

Putnam (386) 326-3200

Santa Rosa (850) 983-5200

Sarasota (941) 861-2914

Seminole (407) 665-3281

St. Johns (904) 825-5055

St. Lucie (772) 462-3800

Sumter (352) 793-2701

Suwannee (386) 362-2708

Taylor (850) 584-5087

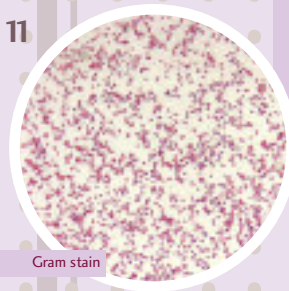
Union (386) 496-3211

Volusia (386) 274-0569

Wakulla (850) 926-3591

Walton (850) 892-8027

Washington (850) 638-6240



Gram stain

Francisella tularensis (Tularemia)

BSL-3 agent Procedures should be performed in a lab that utilizes Biological Safety Level 2 (BSL-2) practices and a biological safety cabinet (BSC) is highly recommended. This organism is highly virulent and it should NOT be manipulated at the bench!

Because of the highly infectious nature of this organism (BSL-3) the state public health

laboratory/department should be consulted immediately if tularemia is suspected.

SPECIMEN COLLECTION

Clinical Samples

BLOOD CULTURES Collect appropriate blood volume and number of sets per established laboratory protocol. †

BIOPSIED TISSUE OR SCRAPING OF AN ULCER Preferable; a swab † of the ulcer is an acceptable alternative. Obtain a firm sample of the advancing margin of the lesion.

ASPIRATE OF INVOLVED TISSUE

AUTOPSY SPECIMEN Include capped needle/syringe used to collect aspirate. Submit tissue, scraping or aspirate in a sterile container. For small tissue samples, add several drops of sterile normal saline to keep the tissue moist.

TRANSPORT/STORAGE OF SPECIMENS TO LABORATORY

BLOOD Transport directly to lab at RT.

BIOPSY/ASPIRATE Transport at RT for immediate processing. If processing is delayed, keep specimen chilled at 2–8°C.

SWABS If using a swab transport carrier, the swab should be reinserted into the transport package and the swab fabric moistened with the transport medium inside the packet. Transport at 2–8°C; room temperature is acceptable. If processing is delayed, keep specimen chilled at 2–8°C.

† Before specimen collection, please refer to pg. 21.

Francisella tularensis (Tularemia)

SENTINEL (LEVEL A) LABORATORY TESTS

GRAM STAIN Tiny (0.2–.5µm X 0.7–1.0µm) poorly staining Gram negative coccobacilli from blood, lymph node aspirate or respiratory specimens

Organism is smaller than Haemophilus influenzae.

GROWTH CHARACTERISTICS IN BROTH

GROWTH CHARACTERISTICS ON AGAR Blood isolates that grow slowly on chocolate agar but poorly on blood agar; robust growth on BCYE agar, organism requires cysteine. Organism will not grow on MacConkey or EMB agar. Plates are held for 5 days or 7 days if treated with antibiotics.

OXIDASE NEGATIVE

CATALASE WEAK POSITIVE

BETA-LACTAMASE POSITIVE

XV TEST NEGATIVE

UREASE TEST NEGATIVE

Not part of Level A routine procedures. The identification of F. tularensis should not be attempted by Level A laboratories using commercial identifications systems because of the potential of generating aerosols and the high probability of misidentification.

REFER TO REFERENCE LABORATORY

CONFIRMATION TESTING (DFA, Slide Agglutination and Antibody Serology, TRF)

Culture and PCR

ISOLATE (plate or slant) at RT

WHOLE BLOOD 1 ml, chilled

Serology

SERUM (separated), 0.5 ml, chilled (NOT frozen)

CITRATED PLASMA (separated), 0.5 ml, chilled (NOT frozen)

Stains

ISOLATE (plate or slant) at RT

SWAB † from affected area or tissues (ulcers, aspirates, lymphoid tissue, blood, bronchial washings/transtracheal aspirate, sputum smear)

TRF

ISOLATE (plate or slant) at RT

SWAB † specimens from ulcer, aspirate, lymphoid tissue, serum, nasopharyngeal or bronchial washings/transtracheal aspirate, chilled

† *Before specimen collection, please refer to pg. 21.*

Francisella tularensis (Tularemia)

Autopsy

Samples from visible abscesses, scrapings from lymph node, lung, liver, spleen, CSF, bone marrow. Transport at RT for immediate processing. If processing is delayed, keep specimen chilled at 2–8°C.

SHIPPING REQUIREMENTS: SPECIMENS SENT FROM SENTINEL LAB TO REFERENCE LAB

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Shippers declaration of dangerous goods must be completed
If the reference lab confirms the presence of a Select Agent then all samples, clinical specimens, and isolates stored at the sentinel lab (your facility) must be transferred to the approved reference lab immediately. In addition, CDC form 0.1318 must be filled out by both the sentinel (your facility) and reference lab and sent to CDC within 7 days. ●

Variola major (Smallpox)



Electron microscopy

BSL-4 agent DNA virus with dumbbell shaped core.

SPECIMEN COLLECTION

For collection kits and specimen handling, call your local county health department for assistance. Any suspected case of smallpox MUST be reported.

Collection for vesicles or pustules

USE SCALPEL (OR A STERILE 26 GAUGE NEEDLE) to open, and remove, the top of the vesicle or pustule and place the skin of the vesicle top into a sterile/dry 1.5–2ml screw capped plastic tube; parafilm cap. Leave the material dry. Label the tube. †

SCRAPE THE BASE OF THE VESICLE OR PUSTULE with the blunt edge of the scalpel, or with the wooden end of an applicator stick or swab and smear the scrapings on to a glass microscope slide. Use a minimum of 3 slides avoiding RBC contamination.

- In addition, apply a microscope slide to the vesicular fluid multiple times, with progressive movement of the slide, to make a touch prep. Use a minimum of 3 slides avoiding RBC contamination.
- Allow the fluid to air-dry 10 minutes, without smearing. Label the slide. †
- Store the dried slide in a plastic slide container/holder for shipping. Parafilm may be used to wrap the slide container/holder to prevent accidental opening.
- Store slides from different patients in separate plastic slide holders to prevent cross contamination.
- If a slide is not available, swab the base of the lesion with a polyester or dacron swab †, place in a screw-capped plastic vial, break off applicator handle, and screw on lid. **DO NOT** add transport medium to the vial. Label the container. †
- If available, lightly touch an electron microscope grid to the unroofed base of the lesion and allow to air dry. Repeat this procedure 2 more times, varying the pressure applied to the unroofed lesion (lighter, or firmer pressure). Place in gridbox and record which slot is used for each patient specimen. Seal gridbox with parafilm or tape. Label the gridbox. †

† *Before specimen collection, please refer to pg. 21.*

BIOPSY VESICLES (2) with 3.5 or 4 mm punch biopsy kit

- Place 1 biopsy in a 1.5–2ml screw capped container with formalin (close tightly and seal with parafilm)
- Place 1 biopsy in a 1.5–2ml screw capped container (parafilm cap)-do not add any fluid
- Label the containers † (indicate if preservative)

COLLECTION OF BLOOD TUBES *see below****Collection for scab lesions**

USE A 26 GAUGE NEEDLE to pick/pry off scabs (at least 4). Place 2 scabs in each of 2 screw-capped plastic 1.5–2ml vials. Label the vials †

BIOPSY LESIONS (2) with 3.5 or 4 mm punch biopsy kit

- Place 1 biopsy in formalin in a 1.5–2ml screw capped container (close tightly and parafilm)
- Place 1 biopsy in a 1.5–2ml screw capped container-do not add any fluid
- Label the containers † (indicate if preservative)

COLLECTION OF BLOOD TUBES *see below*****Collection of blood tubes****FOR PATIENTS WITH VESICLES/PUSTULES OR SCAB LESIONS**

- Draw 10 ml of blood into a plastic marble-topped tube, or a plastic yellow-topped serum separator tube. (If plastic tubes not available, use glass tube using styrofoam protector for shipping). Label the tube. †
- Additional 5ml of blood into a plastic purple top tube may be collected for the CDC for viral culture. Gently shake the tube containing the blood to mix the tube contents and prevent clotting of blood. Label the tube. † (If plastic tubes not available, use glass tube using styrofoam protector for shipping).

Autopsy

SPECIMENS FOR VIRAL ISOLATION SHOULD BE FROZEN (shipped on dry ice), including portions of skin containing lesions, liver, spleen, lung, lymph nodes and/or kidney.

PCR-TISSUE SPECIMEN IN PBS, chilled/frozen

FORMALIN-FIXED TISSUE IS SUITABLE FOR HISTOPATHOLOGY AND

IMMUNOHISTOCHEMISTRY BUT SHOULD NOT BE FROZEN and must be packaged separately from autopsy specimens for virus isolation (which must be frozen). All major organs (liver, spleen, skin, lung, lymph nodes, and/or kidney) should be adequately sampled and submitted for evaluation. Label specimens. †

† *Before specimen collection, please refer to pg. 21.*

TRANSPORT/STORAGE OF SPECIMENS

Do **NOT** send specimens via pneumatic tube system.

SPECIMENS FROM A SINGLE PATIENT place into a Ziploc type biohazard bag with an outside label that includes:

- Patient name
- Date of collection
- Social security number or date of birth of patient

PACKAGE SPECIMENS FROM A SINGLE PATIENT (EXCEPT BIOPSIES):

- On gel packs at 4°C
- In appropriate bio-safety shipping containers in a manner to withstand all shocks, pressure changes, or other conditions incident to ordinary handling in transportation
- In a manner to avoid leakage of contents

PACKAGE NON-FORMALIN LESION BIOPSY FOR SHIPPING on dry ice, leave formalin fixed biopsy at RT. **DO NOT FREEZE** formalin fixed biopsy sample.

SPECIMENS MAY BE STORED IN CONDITIONS OUTLINED ABOVE if shipped within 24 hours of collection. If this is not possible, store samples; except for electron microscope grids, and serum which should remain at 4°C; on dry ice or at -20°C–70°C until, and through, shipment. If there will be a delay in shipping, spin serum to separate from clot, store at 4°C; and ship at 4°C.

FINAL INSTRUCTIONS regarding transportation will be given at the time of consultation and may involve an escort carrier to ensure tracking and integrity.

SENTINEL (LEVEL A) LABORATORY TESTS

Level A laboratory procedures are designed to ensure the proper collection and distribution of appropriate specimens to designated testing laboratories.

REFER TO REFERENCE LABORATORY

✦ *Before specimen collection, please refer to pg. 21.*

SHIPPING REQUIREMENTS: SPECIMENS SENT FROM SENTINEL LAB TO REFERENCE LAB

Contact your DOH state laboratory director if you represent a laboratory that would like to ship a biologic agent to an approved advanced capacity laboratory for presumptive or confirmatory identification. Inform the DOH State Laboratory Director as to the identity of the suspected critical biologic agent.

Follow guidelines for packing and labeling using IATA Packaging Instruction 602 for infectious substances or 650 for diagnostic specimens or DOT 49 CFR 173.196 for infectious substances or 49 CFR 173.199 for diagnostic specimens (using the more restrictive regulation). General packaging requirements for transport of biological agents includes triple (primary receptacle, water tight secondary packaging, durable outer packaging) packaging and requires the “Infectious Substance” label on the outside of the package.

Shippers declaration of dangerous goods must be completed **If the reference lab confirms the presence of a Select Agent** then all samples, clinical specimens, and isolates stored at the sentinel lab (your facility) must be transferred to the approved reference lab immediately. In addition, CDC form 0.1318 must be filled out by both the sentinel (your facility) and reference lab and sent to CDC within 7 days. ●

Viral Hemorrhagic Fever

- Arenaviruses: Lassa, Machupo
- Filoviruses: Ebola, Marburg

BSL-4 agent



Ebola virus—
Electron microscopy

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SPECIMEN COLLECTION

Clinical Samples

SAMPLE FOR SEROLOGY 10–12 ml (min. 5 ml) marble top tube

- Acute (drawn at admission)
- Convalescent (drawn 21 days later)
- Additional 5ml green top and purple top tubes may be collected for the CDC for viral culture and PCR testing, chilled. Gently shake the tube containing the blood to mix the tube contents and prevent clotting of blood.
- Label the tubes. †

TISSUE

TISSUE FOR IMMUNOHISTOCHEMISTRY (preferred lung, kidney or spleen; others lymph node, heart, pancreas, pituitary, brain and liver)-formalin-fixed or paraffin block (preferred) in a separate sterile screw capped container; close tightly and parafilm

BUFFY COAT AND BLOOD CLOT

TISSUE FOR PCR/VIRUS ISOLATION (fresh frozen; preferred lung, kidney or spleen; others lymph node, heart, pancreas, pituitary, brain and liver) in a separate sterile screw capped container; close tightly and parafilm

ANTE-MORTEM biopsy of lung or bone marrow aspirate or clot (1 cm³) in a separate sterile screw capped container; close tightly and parafilm

LABEL THE CONTAINER † (indicate if preservative)

AUTOPSY

Autopsy/surgical report required

POSTMORTEM draw blood from the heart

POST-MORTEM biopsy of spleen, lung, kidney, liver, lymph node, heart, pancreas, pituitary and brain (1 cm³) in a separate sterile screw capped container; close tightly and parafilm

BUFFY COAT, BLOOD CLOT

LABEL THE CONTAINER †

† Before specimen collection, please refer to pg. 21.

Viral Hemorrhagic Fever

- Arenaviruses: Lassa, Machupo
- Filoviruses: Ebola, Marburg

TRANSPORT/STORAGE OF SPECIMENS

BLOOD SAMPLE Ship on dry ice/Serum-ship cold or on dry ice in a plastic tube

SHIP BLOCKS/TISSUE For immuno-histochemistry at RT; DO NOT FREEZE

TISSUE FOR PCR Ship on dry ice

SENTINEL (LEVEL A) LABORATORY TESTS

Level A laboratory procedures are designed to ensure the proper collection and distribution of appropriate specimens to designated testing laboratories.

REFER TO REFERENCE LABORATORY

CONFIRMATION TESTING (Antigen capture ELISA, IgG ELISA, PCR and virus isolation)

SHIPPING REQUIREMENTS: SPECIMENS SENT FROM SENTINEL LAB TO REFERENCE LAB

Contact your DOH state laboratory director if you represent a laboratory that would like to ship a biologic agent to an approved advanced capacity laboratory for presumptive or confirmatory identification. Inform the DOH State Laboratory Director as to the identity of the suspected critical biologic agent.

Follow guidelines for packing and labeling using IATA Packaging Instruction 602 for infectious substances or 650 for diagnostic specimens or DOT 49 CFR 173.196 for infectious substances or 49 CFR 173.199 for diagnostic specimens (using the more restrictive regulation). General packaging requirements for transport of biological agents includes triple (primary receptacle, water tight secondary packaging, durable outer packaging) packaging and requires the "Infectious Substance" label on the outside of the package.

Shippers declaration of dangerous goods must be completed **If the reference lab confirms the presence of a Select Agent** then all samples, clinical specimens, and isolates stored at the sentinel lab (your facility) must be transferred to the approved reference lab immediately. In addition, CDC form 0.1318 must be filled out by both the sentinel (your facility) and reference lab and sent to CDC within 7 days. ●

✦ *Before specimen collection, please refer to pg. 21.*

Supplies	Bacillus anthracis	Yersinia pestis	Clostridium botulinum	Francisella tularensis	Variola Major	VHF
Blood culture bottles	•	•		•		
Red-topped blood collection tube (plastic)	•		•	•		•
Marble-topped blood collection tube (plastic)	•	•	•	•	•	•
Yellow-topped serum separator tube (plastic)					•	
Purple-topped (EDTA) blood collection tube (plastic)	•	•			•	•
Green-topped (Heparin) blood collection tube (plastic)						•
Blue-topped (Citrate) blood collection tube (plastic)	•	•		•		
Plastic slide container/holder					•	
Glass microscope slides frosted end (3x1 inch) 1.22mm thick					•	
Sterile swabs (wood shaft)					•	
Naso-pharyngeal swab	•	•				
Swabs-Dacron or polyester foam (bacterial culturette)	•	•	•	•		
Swabs-Dacron or polyester foam (viral culturette)					•	
Anaerobic Swabs			•			•
Stool collection container	•		•			•
Cary-Blair transport media (stool specimen)	•					
Parafilm					•	•
Sterile screw capped container (large)-tissue	•	•	•	•	•	•
Sterile screw capped container (small)	•	•	•	•		
Sterile/Dry 1.5-2ml screw capped plastic tube					•	
Enema Kit			•			
Port-A-Cult Vials (Becton Dickinson)			•			
Capped needle syringe		•		•		•
Smallpox Collection Kit					•	
Scalpel	•	•	•	•	•	•
3.5 mm or 4.0mm Punch Biopsy Kit					•	•
Formalin					•	•
26 gauge needle					•	•
PBS-phosphate buffered saline		•		•	•	•

Labeling Samples:

1. Patient name and unique identification number (written in pencil for slides)
2. Indicate on specimen container if a preservative is being used; i.e. formalin
3. Place each tissue sample in separate containers and label with name of tissue

Specimen Collection:

- It is recommended to collect specimens before prophylaxis is administered.
- Routine blood culture collection is times two from different collection sites with no more than two sets in a 24-hour period.
- Dacron type swabs or polyester foam requested. Use appropriate bacterial or viral culturette; bacterial culturette for collection of Anthrax, Plague, Botulism and Tularemia specimens and viral culturette for smallpox and viral hemorrhagic fever specimens.
Do not use wooden shaft swabs except for small pox lesion scrapings.

Paperwork accompanying specimen MUST include:

- Unique identification number; will correlate with identity of source if multiple specimens collected
- Source of specimen (complete description; i.e. vesicle, pustule, scab and location of specimen i.e. right leg, left eyelid)
- Date of collection
- Social Security number or date of birth of patient
- Name or initials of person collecting specimen
- If hospitalized, include hospital identification number (e.g. surgical path #)

Rejection Criteria:

- Use established laboratory criteria
- Dried specimens should be referred to your state public health laboratory
- Environmental/nonclinical samples and samples from announced events are not processed by Level A laboratories; submitter should contact the state public health laboratory directly
- Incomplete documentation: All specimens must include the sender's name and telephone number to contact for the preliminary report and additional information
- Improper packaging/shipping
- Do not ship specimens to higher-level LRN laboratories without prior approval

Abbreviations:

BCYE	buffered charcoal-yeast extract agar
BSL	biological safety level
BSL2	biosafety level 2: moderate-risk agents present in the community; disease of varying severity
BSL3	biosafety level 3: indigenous or exotic agents, aerosol transmission; serious and potentially lethal infection
BSL4	biosafety level 4: dangerous or exotic, high risk agents; life threatening disease
CDC	Center for Disease Control and Prevention
cm	centimeters
CSF	cerebral spinal fluid
DOH	Florida Department of Health
DOT	Department of Transportation
DFA	direct fluorescent antibody
EMB	eosin methylene blue agar
IATA	International Air Transport Association
ml	milliliters
mm	millimeters
PBS	phosphate buffered saline
PCR	polymerase chain reaction
RBC	red blood cell
RT	room temperature
SBA	sheep blood agar
TRF	time resolved fluorescence

Definitions:

Reference Laboratory: confirmation testing facilities (state laboratory or CDC)

Sentinel Laboratory: public and private clinical laboratories designed to recognize, rule out and refer specimens for confirmation testing

FLORIDA DEPARTMENT OF
HEALTH

FIDI
Florida Infection
Disease Institute

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