

Guide D

Specimen Collection and Transport Guidelines

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In the United States and its territories, a suspected case of smallpox must be immediately reported to the appropriate local, state or territorial health department. After their review, if smallpox is still suspected, the case should be immediately reported to one of the following:

- 1) **Poxvirus Section, Division of Viral and Rickettsial Diseases, NCID, CDC, Atlanta, Georgia 30333 (phone: Laboratory 404-639-4931, Branch 404-639-3532, Division 404-639-3574, or Center 404-639-3311) – (8am to 5pm, weekdays)**
- 2) **Bioterrorism Preparedness and Response Program, NCID, CDC (404-639-0385, or 404-639-2468) – (8am to 5pm weekdays)**
- 3) **Emergency Preparedness and Response Branch, NCEH, CDC, (770-488-7100) – (any time)**

CDC has appropriate secure containment facilities for storing and working with variola virus and appropriate laboratories to safely evaluate for other human poxviruses.

Approval must be obtained prior to the shipment of potential smallpox patient clinical specimens to CDC. After contacting the CDC (see above) for approval to send the specimens, the samples should be collected in the manner outlined below and placed in an appropriate bio-safe shipping container (see below for IATA Guidelines for Packaging and Transporting Biological Specimens). Specimens collected from patients with poxvirus infections can be stored at 4 °C for a short time, but -20 °C to -70 °C (dry ice) should be used for shipping and long-term storage. Changes in the pH of specimens, such as those caused when dry-ice vapors enter containers, can be avoided by sealing vials with Parafilm and using proper closures with a gasket. **Specific directions regarding the transportation method for the packaged specimens to CDC will be given at the time of consultation.**

For variola virus testing at CDC, it is extremely important not to cross-contaminate samples (i.e., one sample per container) and to collect an amount of lesion specimen sufficient to permit effective testing, by electron microscopy, cell culture, and PCR.

Specimen Collection Supply List

Some or all of the following materials will be required for specimen collection from each patient:

- Disposable protective latex or vinyl gloves (sterile gloves not required)
- Disposable protective gowns
- N-95 masks or higher properly-fitted HEPA-filtered respirators (see below)
- Protective eyewear
- Biohazard plastic disposable bags

- 1 disposable scalpel with No. 10 blade
- Several sterile 26-gauge needles
- 1 3.5- or 4-mm punch biopsy kit
- needle driver
- suture
- suture removal kit
- 4 to 8 sterile dry polyester or cotton swabs
- 4 clean plastic or glass microscope slides
- 4 plastic single-slide holders
- 2 or more electron microscopy grids
- electron microscopy quality forceps
- electron microscopy grid box
- 8 1.5- to 2.0-ml sterile screw-capped plastic vials (Sarstedt with o-ring)
- 5- or 10-cc syringe with 18- or 20-gauge needle
- 1 vacutainer holder
- 2 vacutainer needles (20 x 1 ½ in.)
- 1 10-cc marble-topped vacutainer tubes, **or** 1 10-cc yellow-topped serum separator tube for serum collection (plastic tube preferable)
- 1 5-cc purple-topped tubes for whole blood buffy coat collection for viral isolation (plastic tube preferable)
- Parafilm

Only recently, successfully vaccinated personnel (within 3 years) wearing appropriate barrier protection (gloves, gown, and shoe covers) should be involved in specimen collection for suspected cases of smallpox. Respiratory protection is not needed for personnel with recent, successful vaccination. Masks and eyewear or face shields should be used if splashing is anticipated.

If unvaccinated personnel must be utilized to collect specimens, only those without contraindications to vaccination should be utilized as they would require immediate vaccination if the diagnosis of smallpox is confirmed. Fit-tested N95 masks should be worn by unvaccinated individuals caring for suspected patients.

I. Specimen collection procedure for patients with vesicles or pustules

Blood samples from person with severe, dense rash may be difficult to draw as the skin may slough off. A central line may be needed for access in cases where a peripheral blood draw is difficult.

1. Put on protective equipment described above
2. 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 1.5- to 2-mL screw capped plastic tube. Leave the material dry. **Label the tube as outlined below.**

3. 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 do the following:
 - a. Apply a microscope slide to the vesicular fluid multiple times, with progressive movement of the slide, to make a touch prep.
 - b. Allow the fluid to air-dry 10 minutes without smearing.
 - c. **Label the slide as outlined below**
 - d. Store the dried slide in a plastic slide container
 - e. Store slides in plastic slide holders for shipping. Parafilm may be used to wrap the slide holder to prevent accidental opening. Store slides from different patients in separate plastic slide holders to prevent cross contamination
 - f. If a slide is not available, swab the base of the lesion with a polyester or cotton 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 as outlined below.**
4. If available, lightly touch an electron microscope grid to the unroofed base of the lesion and allow to air dry. Repeat this procedure two 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 .
5. Biopsy vesicles (2) with 3.5- or 4-mm punch biopsy kit.
 - a. Place one biopsy in formalin
 - b. Place one biopsy in a 1.5- to 2-mL screw-capped container – do not add any fluid.
 - c. Label the containers as outlined below
6. Draw 10 cc of blood into a plastic marble-topped tube, or a plastic yellow-topped serum separator tube. **Label the tube as outlined below** and place in collection bag.

If plastic tubes are not available:

 - a. Draw blood into a glass marble-topped or yellow-topped serum separator tube
 - b. Label glass tube as outlined below and place glass tube into a Styrofoam protector for packaging and shipping.
7. Test in Validation: Swab or brush posterior tonsillar tissue, then break off end of applicator into a 1.5- to 2-mL screw-capped tube. Do not add transport medium. **Label the tube as outlined below.**
8. Test in Validation: Draw 5 cc of blood into plastic purple-topped tube. Gently shake the tube containing the blood to mix the tube contents and prevent clotting of blood. **Label the tube as outlined below** and place in collection bag.

If plastic tubes are not available:

 - a. Draw blood into a glass purple-topped tube.
 - b. Gently shake tube to mix the contents
 - c. Label tube as outlined below and place in Styrofoam protector for packaging and shipping.
9. **Label all samples as follows:**
 - a. Patient name
 - b. date of collection

- c. source of specimen (vesicle, pustule, or scab)
- d. social security number or date of birth of patient (for cross referencing of specimens)
- e. name or initials of person collecting specimen
- f. if patient is hospitalized, include hospital identification number (e.g. surg path #)

10. Place specimens from a single patient into a biohazard bag with an outside label that includes:

- a. Patient name
- b. Date of collection
- c. Social security number or date of birth of patient

11. Package specimens from a single patient (except biopsies):

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

12. Package non-formalin lesion biopsy for shipping on dry ice, leave formalin fixed biopsy at room temperature. DO NOT FREEZE formalin fixed biopsy sample.

13. 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 to -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.

14. Final instructions regarding transportation will be given at the time of consultation and may involve a personal escort carrier to ensure sample tracking and integrity.

15. After specimen collection is completed, all protective materials worn by the specimen collector (gloves, mask, gown, etc.) and all used sample collection materials (vacutainer holders, swabs, etc.) must be placed in red biohazard bags and autoclaved or incinerated prior to disposal. Needles should be disposed of in an appropriate sharps container.

II. Specimen collection procedure for patients with scab lesions

1. Put on protective equipment as outlined above and use a 26-gauge needle to pick/pry off as many scabs as possible (at least four).
2. Place two scabs in each of two screw-capped plastic 1.5- to 2-mL vials.
3. Biopsy lesions (2) with 3.5- or 4-mm punch biopsy kit.
 - a. Place one biopsy in formalin
 - b. Place one biopsy in a 1.5- to 2-mL screw-capped container.
 - c. Label the containers as outlined below
4. Draw 10cc of blood into a plastic marble-topped tube, or a plastic yellow-topped serum separator tube. **Label the tube as outlined below** and place in collection bag.

If plastic tubes are not available:

- c. Draw blood into a glass marble-topped or yellow-topped serum separator tube
 - d. Label glass tube as outlined below and place glass tube into a Styrofoam protector for packaging and shipping.
- 5. **Test in Validation:** Swab or brush posterior tonsillar tissue, then break off end of applicator into a 1.5- to 2-mL screw capped tube. Do not add transport medium. **Label the tube as outlined below.**
- 6. **Test in Validation:** Draw 5 cc of blood into plastic purple-topped tube. Gently shake the tube containing the blood to mix the tube contents and prevent clotting of blood. **Label the tube as outlined below** and place in collection bag.
 - If plastic tubes are not available:
 - a. Draw blood into a glass purple-topped tube.
 - b. Gently shake tube to mix the contents
 - c. Label tube as outlined below and place in Styrofoam protector for packaging and shipping.
- 7. **Label all samples as follows:**
 - a. Patient name
 - b. Date of collection
 - c. Source of specimen (vesicle, pustule, or scabs)
 - d. Social security number or date of birth of patient (for cross referencing of specimens)
 - e. Name or initials of person collecting specimen
 - f. If patient is hospitalized, include hospital identification numbers (e.g. surg-path #).
- 8. **Place specimens from a single patient into a biohazard bag with an outside label that includes:**
 - a. Patient name
 - b. Date of collection
 - c. Social security number or date of birth of patient
- 9. Package specimens from a single patient (except for biopsies):
 - a. On gel packs at 4 °C
 - b. In appropriate bio-safety shipping containers in a manner to withstand all shocks, pressure changes, or other conditions incident to ordinary handling in transportation
 - c. In a manner to avoid leakage of contents
- 10. Package nonformalin lesion biopsy for shipping on dry ice; package formalin biopsy at room temperature. **DO NOT FREEZE** formalin-fixed biopsy sample.
- 11. Specimens may be stored in conditions outlined above if shipped within 24 hours of collection. If this is not possible, store samples, except for serum and formalin fixed biopsy, on dry ice or at -20 °C to -70 °C. If there will be a delay in shipping, spin serum to separate from clot, store at 4 °C, and ship at 4 °C.
- 12. Final instructions regarding transportation will be given at the time of consultation and may involve a personal escort carrier to ensure sample tracking and integrity.
- 13. After specimen collection is completed, all protective materials worn by the specimen collector (gloves, mask, gown, etc.) and all used sample collection

materials (vacutainer holders, swabs, etc.) must be double-bagged in red biohazard bags and autoclaved or incinerated prior to disposal. Needles should be disposed of in an appropriate sharps container.

III. Autopsy specimens[†]

1. Autopsy specimens for virus isolation should be frozen (shipped with dry ice), including portions of skin containing lesions, liver, spleen, lung, lymph nodes, and/or kidney
2. Formalin-fixed tissue is suitable for histopathology, immunohistochemistry and PCR 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.
3. Specimens should be labeled and packaged for transport as outlined above.
4. After specimen collection, all non-reusable specimen collection and barrier protection materials should be placed in red biohazard bags and autoclaved prior to disposal. All reusable autopsy equipment must be autoclaved or disinfected according to standard laboratory procedures before re-use.

[†] Extreme precautions are necessary to prevent dissemination of smallpox virus during an autopsy. Standard Precautions should be observed for all contact with the body. Contact with the CDC (NCID Division of Healthcare Quality Control at 404-639-6413 or Pathology Activity 404-639-3133) should be made, prior to an autopsy, in order to review the containment features of individual autopsy suites, procedures for autopsy, and disinfection after an autopsy. To transport the body to the autopsy suite, the body should be wrapped in a large, impervious plastic bag, or a disaster pouch, that is sealed airtight with tape. The body should be sealed in a second large, impervious plastic bag prior to transportation to the autopsy suite. Ideally, the autopsy would be performed in a room with negative air pressure with respect to the surrounding facilities. All doors and windows of the autopsy rooms should be closed during the autopsy, and the air exhausted must not be recirculated. Only necessary personnel with up-to-date vaccination (within 3 years) should participate in the autopsy. Vaccinated personnel should wear disposable clothing, gowns, gloves, caps, booties, and masks and face shields or protective eyewear to prevent splashing of the mucus membranes. No personal clothing should be worn. All clothing articles from the autopsy room should be placed in biohazard bags and autoclaved or incinerated. After autopsy, the body should be double-bagged [see above] as described above, in another set of large, impervious plastic bags. If vaccination prior to autopsy is not possible, unvaccinated personnel should perform the autopsy wearing, in addition to the protective garments above, respiratory protection (e.g., HEPA-filtered breathing apparatus or a self-contained breathing apparatus).

****Avoid glass vials if possible. Use plastic vials, bottles, or slide holders as the primary container for ALL specimens.**

***** Each patient's lesion specimens must be packaged separately from other patient specimens to avoid cross-contamination.**

All procedures involving handling potentially infectious material should be performed in laboratories utilizing Biosafety Level 2 or 3 practices. Any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, or applying makeup should be prohibited. Thorough hand-washing using soap or soap containing Lysol or soaps such as *Hibiclens* should be done prior to leaving the laboratory. Areas of the skin known to have come in contact with virulent variola (or monkeypox) virus should be washed with soap and decontaminated with 0.5% sodium hypochlorite with at least a 1 min contact time. Administration of smallpox vaccination, and possibly VIG should be determined in coordination with CDC.

In the event of a large outbreak of confirmed smallpox, other laboratories with smallpox diagnostic capabilities may be used for diagnostic surge capacity. These laboratories will be designated by CDC and instructions for sending specimens to these laboratories will be given at the time of their designation.

IATA Guidelines for Packaging and Transporting Biological Agents

Biological agents include infectious agents of humans, plants, and animals, as well as the toxins that may be produced by microbes and by genetic material potentially hazardous by itself or when introduced into a suitable vector. Etiologic agents and infectious substances are closely related terms that are found in the transfer and transportation regulations. Biological agents may exist as purified and concentrated cultures but may also be present in a variety of materials such as body fluids, tissues, soil samples, etc. Biological agents and the materials that are known or suspected to contain them are recognized by federal and state governments as hazardous materials and their transportation and transfer is subject to regulatory control.

Transportation refers to the packaging and shipping of these materials by air, land, or sea, generally by a commercial conveyance. Transfer refers to the process of exchanging these materials between facilities.

Transportation

Regulations on the transportation of biological agents are aimed at ensuring that the public and the workers in the transportation chain are protected from exposure to any agent that might be in the package. Protection is achieved through (a) the requirements for rigorous packaging that will withstand rough handling and contain all liquid material within the package without leakage to the outside, (b) appropriate labeling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package, (c) documentation of the hazardous contents of the package should such information be necessary in an emergency situation, and (d) training of workers in the transportation chain to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

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. 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. Mailability of 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>

Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030. Occupational Exposure to Bloodborne Pathogens. Provides minimal packaging and labeling requirements for transport of blood and body fluids within the laboratory and outside of it. Information may be obtained from your local OSHA office or from the Internet:
<http://osha.gov>

Dangerous Goods Regulations (DGR). International Air Transport Association (IATA). These regulations provide packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These are the regulations followed by the airlines. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air that is provided by the International Civil Aviation Organization (ICAO). A copy of the DGR may be obtained by calling 1-800-716-6326 or through the Internet at:
<http://www.iata.org>, or <http://www.who.org>

General Packaging Requirements for Transport of Biological Agents and Clinical Specimens

Figure 1 shows the generalized "triple" (primary receptacle, water tight secondary packaging, durable outer packaging) packaging required for a biological agent of human disease or materials that are known or suspected of containing them. This packaging requires the "Infectious Substance" label shown in Figure 2 on the outside of the package. This packaging must be certified to meet rigorous performance tests as outlined in the DOT, USPS, PHS, and IATA regulations.

Clinical specimens with a low probability of containing an infectious agent are also required to be "triple" packaged, but performance tests require only that the package shall not leak after a four-foot drop test. DOT, PHS, and IATA require a "clinical specimen" label on the outside of the package.

Transfer

Regulations on the transfer of biological agents are aimed at ensuring that the change in possession of biological materials is within the best interests of the public and the nation. These regulations require documentation of the personnel, facilities, and justification of need for the biological agent in the transfer process and subsequent approval of the transfer process by a federal authority. The following regulations fit in this category:

Importation of Etiologic Agents of Human Disease

42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiologic Agents, Hosts and Vectors. This regulation requires an import permit from the Centers for Disease Control and Prevention for importing etiologic agents of human disease and any materials, including live animals or insects, that may contain them. An application and information on importation permits may be obtained by calling 1-888-CDC-FAXX and enter document number 101000 or on the Internet at: <http://www.cdc.gov/od/ohs/biosfty/impptper.htm>

Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases

9 CFR Parts 92, 94, 95 96, 122 and 130. These regulations requires an import permit from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services to import or domestically transfer etiologic agents of livestock, poultry, other animals, and any materials that might contain these etiologic agents. Information may be obtained at (301) 734-3277, or from the Internet at: <http://aphisweb.aphis.usda.gov/ncie>

Transfer of Select Biological Agents of Human Disease

42 CFR Part 72.6 Additional Requirements for Facilities Transferring or Receiving Select Agents. Facilities transferring or receiving select agents must be registered with the CDC and each transfer of a select agent must be documented. Information may be obtained on the Internet at: <http://www.cdc.gov/od/sap/>

Export of Etiologic Agents of Humans, Animals, Plants and Related Materials

Department of Commerce. 15 CFR Parts 730 to 799. This regulation requires that exporters of a wide variety of etiologic agents of human, plant and animal diseases, including genetic material, and products which might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the Internet at: <http://bxa.fedworld.gov> or <http://www.bxa.doc.gov>

Figures 1 and 2 illustrate the packaging and labeling of infectious substances and clinical specimens in volumes of less than 50 ml. in accordance with the provisions of subparagraph 72.3(a) of the regulation on Interstate Shipment of Etiologic Agents (42 CFR, Part 72). A revision is pending that may result in additional package labeling requirements, but this has not been issued in final form as of the publication of this fourth edition of BMBL.

For further information on any provision of this regulation contact:

Centers for Disease Control and Prevention

Attn: External Activities Program

Mail Stop F-05

1600 Clifton Road N.E.

Atlanta, GA 30333

Telephone: (404) 639-4418

FAX: (404) 639-2294

Note that the shipper's name, address and telephone number must be on the outer and inner containers. The reader is also advised to refer to additional provisions of the Department of Transportation (49 CFR, Parts 171-180) Hazardous Materials Regulations.

Shipping suspected biological threat agents to the CDC:

Label the package as follows:

Centers for Disease Control and Prevention

1600 Clifton Road, NE

ATTN: DASH (forward to RRAT Lab)

Atlanta, GA 30333

For shipping questions relating to sending specimens to the CDC, contact **(404) 639-2888**.

Figure 1. Packing and Labeling of Infectious Substances



Figure 2. Packing and Labeling of Clinical Specimens

