

SENTINEL LEVEL CLINICAL LABORATORY GUIDELINES
FOR
SUSPECTED AGENTS OF BIOTERRORISM
AND
EMERGING INFECTIOUS DISEASES

Smallpox

American Society for Microbiology (ASM)

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PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE

Smallpox is a serious, contagious, and sometimes fatal infectious disease. There is no specific treatment for smallpox disease, and the only prevention is vaccination. 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.

There are two clinical forms of smallpox. Variola major is the severe and most common form of smallpox, with a more extensive rash and higher fever. There are four types of variola major smallpox: ordinary (the most frequent type, accounting for 90% or more of cases); modified (mild and occurring in previously vaccinated persons); flat and hemorrhagic (both rare and very severe). Historically, variola major has an overall fatality rate of about 30%; however, flat and hemorrhagic smallpox cases usually are fatal. Variola minor is a less common presentation of smallpox, and a much less severe disease, with death rates historically of 1% or less.

Smallpox is caused by the variola virus that emerged in human populations thousands of years ago. The last case of smallpox in the United States was in 1949. The last naturally occurring case in the world was in Somalia in 1977. After the disease was eliminated from the world, routine vaccination against smallpox among the general public was stopped because it was no longer necessary for prevention.

Except for laboratory stockpiles, the variola virus has been eliminated. However, in the aftermath of the events of September and October, 2001, there is heightened concern that the variola virus might be used as an agent of bioterrorism. For this reason, the U.S. government is taking precautions for dealing with a smallpox outbreak.

Generally, direct and fairly prolonged face-to-face contact is required to spread smallpox from one person to another. Smallpox can also be spread through direct contact with infected body fluids or contaminated objects such as bedding or clothing. Rarely, smallpox has been spread by virus carried in the air in enclosed settings such as buildings, buses, and trains. Humans are the only natural hosts of variola. Smallpox is not known to be transmitted by insects or animals.

A person with smallpox is sometimes contagious with onset of fever (prodrome phase), but the person becomes most contagious with the onset of rash. At this stage the infected person is usually very sick and not able to move around in the community. The infected person is contagious until the last smallpox scab falls off.

Note: sentinel clinical laboratories do not require registration with the Select Agent Program to conduct diagnostic testing for Select Agents, both Tier I and non-Tier 1. Testing for Select Agents may be performed by laboratories as long as the laboratory destroys any residual specimen and destroys or transfers the confirmed select agent with 7 days of identification. Reporting of all identified Select Agents is still required; laboratories will need to complete Form 4. If the organism is transferred following identification, then the laboratory must also complete Form 2. For further guidance and access to the necessary forms, consult with

your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at www.selectagents.gov.

II. SAFETY CONSIDERATIONS

Consultation with the local or state health department laboratory is recommended as soon as smallpox is suspected.

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.

All manipulations of unfixed material must be carried out within a Class III Biological Safety Cabinet, or within a Class II Biological Safety Cabinet while using BSL-3 practices and safety equipment.

All procedures involving handling potentially infectious material should be performed in laboratories utilizing Biosafety Level 2 or 3 practices. Areas of the skin known to have come in contact with 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 Variola immune globulin (VIG) should be determined in coordination with CDC.

III. MATERIALS

Personal protective equipment and safety:

- latex or nitrile gloves
- Disposable protective gowns
- properly fitted N-95 masks or HEPA-filtered respirators
- Protective eyewear
- Biohazard plastic disposable bags

Specimen collection materials:

- 1 disposable scalpel with No. 10 blade
- Several sterile 26-gauge needles
- 4 to 8 sterile dry specimen collection swabs
- 4 clean plastic or glass microscope slides

- 4 plastic single-slide holders
- 8 1.5- to 2.0-ml sterile screw-capped plastic vials 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.)
- one 10-cc marble-topped Vacutainer tubes, or one 10-cc yellow-topped serum separator tube for serum collection (plastic tube preferable)
- Parafilm

Optional:

- two or more electron microscopy grids
- electron microscopy quality forceps
- electron microscopy grid box

IV. SPECIMEN COLLECTION:

Label all tubes, vials, and microscope slide holders with the following information:

- a. Patient name
- b. Date of birth
- c. Date of collection
- d. Source of specimen (e.g., vesicle fluid, pustule roof, or scab)

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

- a. Patient name
- b. Date of birth
- c. Date of collection
- d. Name or initials of person collecting specimens
- e. Additional unique identifier if available.

Recommended specimens include the following:

Vesicular Material

- a. Sanitize the patient's skin with an alcohol wipe and allow skin to dry.
- b. Open the top of a vesicle or pustule with a scalpel, sterile 26-gauge needle, or slide. Collect the skin of the vesicle top in a dry, sterile 1.5- to 2-mL screw-capped tube. Label the tube.
- c. Scrape the base of the vesicle or pustule with the wooden end of an applicator stick or swab and smear the scrapings onto a glass or plastic light microscope slide. Allow slide to dry for 10 minutes.
- d. Label the slide and place it in a slide holder. To prevent cross-contamination, do not place slides from more than one patient in the same slide holder.
- e. Take another slide, and touch it repetitively to the opened lesion using progressive movements of the slide in order to make a touch prep. Allow slide to dry for 10 minutes.
- f. Label the slides as touch preps and place in the same slide holder. To prevent cross-contamination, do not place slides from more than one patient in the same slide holder. Parafilm may be used to wrap the slide holder to prevent accidental opening.

- g. If plastic-coated electron microscopic (EM) grids are available, lightly touch the shiny side of 3 EM grids to the base of the open lesion, allow EM grids to air-dry for 10 minutes, and place grids in an appropriately labeled grid box. Use varying degree of pressure (minimal, light, and moderately firm) in application of the 3 grids to the unroofed lesion. If a slide or EM grid is not available, swab the base of the lesion with a polyester or cotton swab, place in screw-capped plastic vial, break off applicator handle, and seal.
- h. Repeat this procedure for 2 or more lesions.

Scab Specimens

- a. Sanitize the patient's skin with an alcohol wipe and allow skin to dry.
- b. Use a 26-gauge needle to remove 2 to 4 scabs.
- c. Place 1 or 2 scabs in each of 2 dry, sterile screw-capped plastic tubes.
- d. Wrap parafilm around the juncture of the cap and vial.
- e. Label the tube.

Biopsy Lesions (At least 2 specimens obtained by using a 3.5- or 4-mm punch biopsy kit.)

- a. Use sterile technique and appropriate anesthetic.
- b. Place 1 sample in formalin for immunohistochemical or histopathologic evaluation and store at room temperature.
- c. The second specimen should be placed dry (do not add transport medium) in a sterile 1.5- to 2-mL screw-capped container (do not add transport medium).
- d. Refrigerate if shipment occurs within 24 hours; otherwise, the specimen should be frozen.

Serum

- a. 10 ml of blood into a plastic marble-topped tube, or a plastic yellow-topped serum separator tube. If plastic tubes are not available, draw blood into a glass marble-topped or yellow-topped serum separator tube and place labeled tube into a Styrofoam protector for packaging and shipping.
- b. If possible, centrifuge to separate serum and decant before shipping. Store and ship at refrigeration temperature.

Autopsy specimens

- a. 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
- b. 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.

V. SPECIMEN PROCESSING AND STORAGE:

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.

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 biohazard bags and autoclaved or incinerated prior to disposal. Needles should be disposed of in an appropriate sharps container. All reusable autopsy equipment must be autoclaved or disinfected according to standard laboratory procedures before re-use.

VI. SPECIMEN TRANSPORT

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.

Current Packing and Shipping Guidelines (IATA and DOT) must be adhered to when submitting suspect smallpox specimens to the nearest LRN Reference laboratory.

Select Agent reporting and compliance

1. Reporting of all identified Select Agents is still required, even though Sentinel laboratories are not required to register under the Select Agent Rule.
2. The laboratory must complete Form 2 within one week (7 days) following notification of the confirmed identification. For further guidance and access to the necessary forms, consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at www.selectagents.gov
3. Reporting all identified Select Agents is required by completing Form 4 A within 7 days of confirmed identification. If the isolate is from a Proficiency test sample, Form 4 B is to be completed within 90 days of receipt of the sample.
4. Your designated LRN Reference Laboratory will advise you with completion of required forms (e.g., Forms 2, 3, and 4). Always refer to www.selectagents.gov for the latest guidance and versions of these forms.

ANALYTICAL CONSIDERATIONS

There are no Sentinel laboratory tests to rule out smallpox. Testing for suspect smallpox infection is performed at Laboratory Response Network (LRN) reference laboratories. Tests include generic tests for orthopox, including Non-variola Orthopox PCR, Orthopox PCR, and Electron Microscopy. Variola specific tests include variola culture with PCR confirmation and Variola PCR from clinical specimen. Additional tests may include Vaccinia PCR.

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