Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases

Smallpox (Variola virus)

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ASM Sentinel Level Laboratory Protocol Working Group

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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 prior to sample collection.**

Smallpox is caused by the variola virus that emerged in human populations thousands of years ago and only infects humans (1). 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 and the disease was declared globally eliminated in 1980 (1). The virus only remains in select laboratory stockpiles and unknown stored specimens (2). After the disease was eliminated from circulation, routine vaccination against smallpox among the general public was stopped because it was no longer necessary for prevention. However, with the 2022 surge in another orthopoxvirus, monkeypox virus (MPXV), globally, the vaccinia vaccine previously used for smallpox was administered for its protection against MPXV in high-risk individuals (3, 4). While smallpox is currently eradicated, there is concern about the virus being used as a bioterrorism agent. The probability of a patient having smallpox is very low and other viral infections (e.g., varicella zoster virus and herpes simplex virus) can present similarly and should be considered (5).

Note: sentinel clinical laboratories do not require registration with the Federal Select Agent Program to conduct diagnostic testing for select agents and toxins, both Tier I and non-Tier 1. Testing for select agents may be performed by laboratories with the appropriate biosafety level facilities as long as the laboratory destroys any residual specimen and destroys or transfers the confirmed select agent with seven (7) days of identification.

II. Safety Considerations

Per the United States Centers for Disease Control and Prevention (CDC) guidelines, suspect cases should first undergo risk assessment by Infectious Diseases and/or Dermatology consultation to determine the clinical and public health response. Providers must also notify their institutional Infection Control team and apply appropriate isolation precautions. Consultation with the local or state health department laboratory is required as soon as the patient is determined to be at high risk for smallpox (6). The CDC has recommendations for who should collect the specimens and the biosafety requirements, and they will inform of precautions to be taken. If the patient meets the CDC's high-risk criteria for smallpox, no further specimen testing is permitted until smallpox is ruled out. Note that the CDC advises not to delay treatment for other conditions (6).

If the patient meets the CDC's low- or moderate-risk criteria for smallpox, the laboratory can continue to test for common causes of febrile exanthema (7). All procedures involving handling potentially infectious material should be performed in laboratories utilizing Biosafety Level 2 or 3 practices. All manipulations of unfixed material must be performed within a Class III Biological Safety Cabinet, or within a Class II Biological Safety Cabinet while using BSL-3 practices and safety equipment. Please refer to the CDC Rash Illness Testing Protocol for up-to-date information (7).

III. Specimen Collection, Processing and Storage

Clinicians must contact their local or state public health authorities immediately upon suspecting smallpox and should not collect any specimens before discussing with the appropriate public health authorities. The local or state public health authorities will work with the clinical team to determine if the patient meets the criteria for being considered high-risk for smallpox. If the patient case is deemed to be high-risk, it should be immediately reported to the CDC Emergency Operations Center. Samples from patients who are deemed to be

at low- or moderate-risk can be tested at reference level Laboratory Response Network (LRN) laboratories, while high-risk patient samples are tested at select LRN laboratories and the CDC (5). Public health authorities and the CDC will guide the collection of appropriate specimens based on disease stage and further discuss safety precautions. Specimens may include tonsillar tissue swab, nasopharyngeal swab, lesion biopsy, lesion fluid, lesion fluid swab, lesion fluid smear or touch prep slide, electron microscopy grid, lesion roof, scab or crust, and/or acute/convalescent serum and whole blood. Notably, swabs should not be placed in viral transport media, but submitted as a dry swab in a sterile container with O-ring cap. Specimens, except for formalin-fixed specimens and electron microscopy grids, may be stored at 4°C or -70°C if storage and shipping is anticipated to extend beyond 24 hours after collection (8).

Any potentially infected materials or residual specimens must be placed in biohazard bags and autoclaved or incinerated prior to disposal. Needles or other sharps will be discarded into sharps containers. Biosafety cabinets and other reusable equipment should be decontaminated according to laboratory standard operating procedures.

IV. Considerations For Laboratories Performing Routine Viral Culture

Smallpox should never intentionally be grown in viral culture. However, there is a rare possibility in which laboratories may receive specimens that contain smallpox when the clinician is not suspecting it. While this is extremely unlikely given eradication of the virus, there is always a possibility of a bioterrorism event, so laboratories must be cautious and assume any specimen can contain a highly infectious agent. Below are some characteristics of smallpox when grown in viral culture. If these characteristics are observed, consult the laboratory director and clinical provider to determine if the clinical history is compatible. If unable to rule out the possibility of smallpox, do not discard specimens or inoculated cultures. Contact the CDC for further guidance. Keep in mind that other viruses, especially other orthopoxviruses, may mimic the growth pattern. **Standard methods used by clinical laboratories will not definitively identify variola virus.**

- Variola virus can grow in a wide variety of cell lines including those used for isolation of herpesviruses. Cytopathic effect (CPE) has been best described from Vero, infant foreskin fibroblast, human embryonic lung fibroblast, rhesus monkey kidney, HeLa, and MRC-5 cells (9, 10).
- CPE develops in 1-3 days as rounding of cells, cell fusions and multinucleation foci appear. Clumping of infected cells form small plaques, 1-3 mm (10).
- Hemadsorption is frequently detectable when using human embryonic cell cultures but variable with HEp2 cells (11).

V. Specimen Transport

Final instructions regarding transportation will be given at the time of public health/CDC consultation. High-risk specimens should be packaged, labeled, and shipped as Category A infectious substances affecting humans (UN 2814) while low or moderate-risk specimens should be packaged, labeled, and shipped as Category B infectious substances (UN3373). Regardless of risk, all specimens must adhere to the U.S. Department of Transportation's Hazardous Materials Regulations and the International Air Transport Association (IATA) Dangerous Goods Regulations (5).

VI. Select Agent Reporting and Compliance

Immediate reporting of all identified select agents is still required, even though sentinel clinical laboratories are not required to register under the Select Agent Regulations. The designated LRN reference laboratory will provide guidance on how to complete the required forms (e.g., Forms 2 and 4) and the timeline. The laboratory must complete Form 2 and Form 4A within one week (7 days) following notification of the confirmed identification. For the most up to date guidance, the designated LRN reference laboratory should be consulted, or laboratorians can refer to the Federal Select Agent Program website at www.selectagents.gov.

Analytical Considerations

There are no sentinel laboratory tests to rule out smallpox. Testing for suspected smallpox infection is performed at LRN reference and national laboratories.

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