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**SENTINEL LABORATORY GUIDELINES  
FOR  
SUSPECTED AGENTS OF BIOTERRORISM**

**Unknown Viruses**

American Society for Microbiology

## **Credits: Unknown Viruses**

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## **I. GENERAL INFORMATION**

### **A. Special instructions**

1. If a smallpox virus, filovirus, arenavirus, alphavirus, or Crimean-Congo hemorrhagic fever virus infection is suspected, contact public health officials before specimen collection.
2. DO NOT collect specimens and use viral transport medium unless specifically requested by public health officials.
3. DO NOT accept environmental or animal specimens. Forward such specimens directly to the state health laboratory. Alert public health officials.
4. DO NOT inoculate specimens received for ruling out a potential viral agent of bioterrorism into cell culture. Immediately inform the appropriate personnel designated by the hospital's bioterrorism readiness protocol. Medical personnel should initiate direct communication with the local or state department of health.
5. Unidentifiable patterns of cytopathic effect (CPE) could be caused by a potential agent of bioterrorism. Consult the physician to see if the patient's clinical presentation is consistent with smallpox or a hemorrhagic viral infection. If the patient's history and/or disease is consistent, notify the public health department and follow instructions to forward the culture and remaining specimens to the appropriate laboratory.

### **B. Description of organism**

Viruses described as potential agents of bioterrorism include those listed in Tables 1 and 2 and Fig. 1. These viruses are possible candidates for receipt by the laboratory as an unknown virus if there is an outbreak.

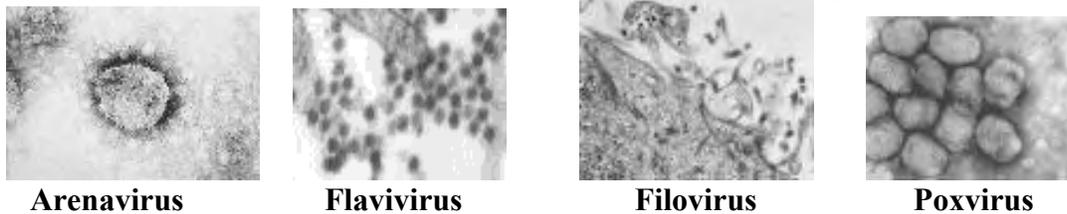
**TABLE 1. Potential viral bioterrorism agents**

<i>Poxviridae</i>	<i>Arenaviridae</i> <sup>a</sup>	<i>Bunyaviridae</i>	<i>Flaviviridae</i>	<i>Filoviridae</i>	<i>Alphavirus</i>
Variola virus <sup>a</sup> (smallpox)	Lassa fever virus	Rift Valley fever virus <sup>a</sup>	Kyasanur Forest disease virus <sup>a</sup>	Ebola virus <sup>a</sup>	Eastern equine encephalitis virus <sup>a</sup>
	Junin virus (Argentine HF <sup>b</sup> )	Crimean-Congo HF virus	Omsk HF virus <sup>a</sup>	Marburg virus <sup>a</sup>	Western equine encephalitis virus
	Machupo virus (Bolivian HF)	Hantavirus (HF)	Dengue HF virus		Venezuelan equine encephalitis virus
	Guanarito virus (Venezuelan HF)		Yellow fever virus		

<sup>a</sup>Viruses with the greatest potential as bioterrorism agents.

<sup>b</sup>HF, hemorrhagic fever.

**FIG. 1. Electron micrographs of various viral bioterrorism agents (<http://www.CDC.gov>)**



**Arenavirus**

**Flavivirus**

**Filovirus**

**Poxvirus**

**TABLE 2. Epidemiology of viral bioterrorism agents**

Virus family	Disease	Geographic location	Transmission	Prevention
<i>Poxviridae</i>	Smallpox	Unknown <sup>a</sup>	Person-person	Vaccine
<i>Arenaviridae</i>	Lassa fever (LF)	West Africa	Rodent	Ribavirin
	South American hemorrhagic fevers (SAHF)	South America	Rodent	Vaccine/ plasma <sup>b</sup>
<i>Bunyaviridae</i>	Crimean-Congo hemorrhagic fever (CCHF)	Asia, Central Africa	Tick	
	Rift Valley fever (RVF)	Africa	Mosquito Blood of infected animals (i.e. sheep)	
	Hantavirus hemorrhagic fever	Southeast Asia	Rodent	
<i>Flaviviridae</i>	Yellow fever	South America, Africa	Mosquito	Vaccine
	Dengue hemorrhagic fever	Asia, Africa, Australia, Americas	Mosquito	Insect repellants (DEET)
	Kyasanur Forest disease	India	Tick	Vaccine
	Omsk hemorrhagic fever	Russia	Tick Muskrat-human	
<i>Filoviridae</i>	Ebola and Marburg hemorrhagic fever (E/MHF)	Africa	Direct contact <sup>c</sup>	
<i>Alphavirus</i>	Eastern equine encephalitis (EEE)	Canada to South America, Caribbean	Mosquito	No human vaccine
	Western equine encephalitis (WEE)	Canada to South America	Mosquito	No human vaccine
	Venezuelan equine encephalitis (VEE)	South America	Mosquito	No human vaccine

<sup>a</sup>Smallpox was deemed eradicated worldwide in 1980.

<sup>b</sup>Vaccine available for Junin virus only. Convalescent-phase plasma therapy useful with some SAHF.

<sup>c</sup>Direct contact with infected body fluids (including vomitus, urine, and stool) promotes transmission of arenaviruses or filoviruses. Airborne transmission is also a remote possibility.

### C. Unknown virus

If a community exposure to a virulent unknown virus occurs, the challenge for the clinical microbiology laboratory is threefold:

1. Management of clinical specimens and/or viral isolates safely and appropriately.
2. Recognition of the agent within the limitations of routine testing.
3. Rapid notification of a potential outbreak to the proper authorities.

A genetically altered or manufactured agent may produce unexpected findings. The documentation of a carefully obtained clinical history provides invaluable information to the physician and the laboratory.

### D. Clinical presentations

#### 1. Smallpox

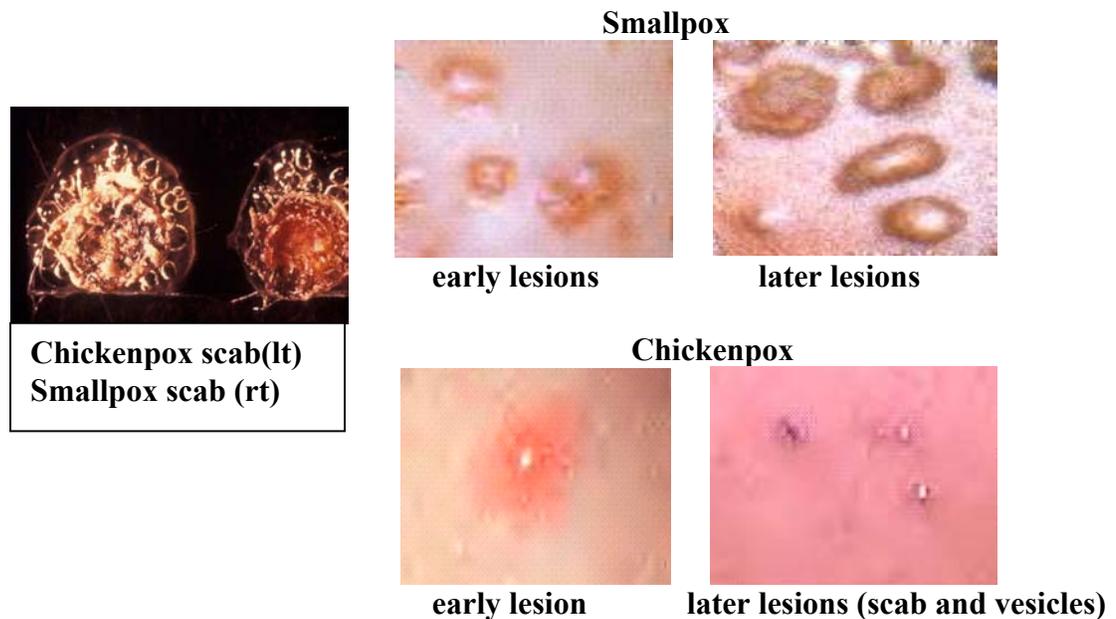
NOTE: An Algorithm for evaluating patients with smallpox and additional information is available from the Centers for Disease Control and Prevention at <http://www.bt.cdc.gov/agent/small-pox/diagnosis/index.asp>

- a. Transmission of variola virus occurs directly by inhalation of infective aerosols or by direct contact with contaminated materials. Viral spread may occur with onset of fever (prodrome phase), but is greatest with the onset of rash. Infectivity diminishes with the onset of scab formation; however, the person is contagious to others until the eradication of all scabs.
- b. Symptoms develop approximately 12 to 14 days after a primary exposure. A period of approximately 2 weeks may ensue before detection of visible symptoms and confirmation of the diagnosis. The symptoms are typical of many viral infections and include fever and severe myalgias.
- c. Initial presentation of vesicles occurs in the oropharynx within 72 h of the first symptoms. Lesions then spread, first to the face and then to the arms, hands, and feet, during the next 7 to 14 days. Vesicles are more abundant on the face, forearms, and lower legs and are sparser on the trunk. The smallpox rash initially presents as erythematous papules which progress to form vesicles, then pustules, and finally scabs. Death may occur within 5 to 7 days in rapidly progressing infection or within 10 to 14 days in the more classical presentation of the infection.
- d. The disease most commonly confused with smallpox is chickenpox. During the first 2 to 3 days of illness, the appearance of the rash of smallpox is indistinguishable from the rash of chickenpox. Several notable differences aid in the correct identification of the viral syndrome: (i) Smallpox lesions develop simultaneously, mature uniformly, and disperse in equal concentrations over infected body sites. In contrast, different developmental stages of lesions (crops) occur concurrently during chickenpox. (ii) Smallpox vesicles are more abundant on the face, forearms, and lower legs and are sparser on the trunk. Chickenpox lesions are more dense over the trunk and never occur on the palms or soles. Lesions often localize on the trunk and head (Fig. 2).
- e. Two severe variations of smallpox also occur. (i) Hemorrhagic smallpox has a shorter incubation period followed by more severe prodromal symptoms and formation of petechiae and hemorrhages into the skin and mucous membranes. Death often occurs within 5 to 6 days of the onset of the petechial rash. The lack of

vesicles and the prominence of cutaneous signs of bleeding may result in the misdiagnosis of hemorrhagic smallpox as meningococemia or acute leukemia. Pregnant women are at increased risk of hemorrhagic smallpox. (ii) Malignant smallpox has an abrupt onset of severe prodromal symptoms. A vesicular rash develops that coalesces into soft, flattened, sometimes hemorrhagic lesions that have the appearance of crepe rubber. The rash does not progress to the formation of pustules or scabs.

- f. Complications arise in some individuals after immunization with the smallpox vaccine. The currently described syndromes are as follows: (i) generalized vaccinia, a harmless infection typified by numerous lesions on the body; (ii) progressive vaccinia (*vaccinia necrosum*) characterized by progressive necrosis of the vaccination site; (iii) *eczema vaccinatum*, an often serious infection that occurs in individuals with eczema; (iv) *erythema multiforme*, an erythematous rash possibly linked to an allergic reaction; and (v) *postvaccinal encephalitis*, a rare central nervous system infection in primary vaccinees (Fig. 3).

**FIG. 2. Comparison of lesions of smallpox and chickenpox (<http://www.CDC.gov>).**



**FIG. 3. Smallpox vaccination lesions (<http://www.CDC.org>).**



## **2. Alphaviruses**

- a.** Most notably recognized as agents of equine encephalitis, the alphaviruses possess the capacity to cause epidemic human disease. In humans as well as horses, the viruses have a proclivity for the central nervous system (CNS).
- b.** Generalized symptoms, such as fever, malaise, and headache, precede the onset of encephalitis. Depending on the etiologic agent, symptoms may progress to more serious neurological manifestations.
- c.** Of the three viral syndromes, Venezuelan equine encephalitis (VEE) has a lower morbidity/mortality rate. Symptoms resembling a flu-like syndrome usually manifest after an incubation period of less than 1 week. Only a small percentage of cases progress to neurological involvement, and the prospects for recovery surpass those for Eastern equine encephalitis virus (EEE) or Western equine encephalitis virus (WEE).
- d.** Both EEE and WEE produce more serious effects that often develop into coma. Fatalities are higher with EEE, and infection often results in permanent sequelae.
- e.** As with all arboviruses, either a mosquito or a rodent vector spreads alphaviruses in nature. Human transmission through infectious aerosols gives these viruses bioterrorism potential.

## **3. Viral hemorrhagic fever (VHF) viruses**

- a.** Clinical symptoms will vary, depending on the virus and response of the patient. After incubation periods ranging from 2 days to 3 weeks, general symptoms include fever, headache, other CNS symptoms, malaise, muscle and joint pain, nausea, diarrhea, and cough. South American hemorrhagic fever (SAHF) virus produces petechiae over the palate and in the axillary regions. The filoviruses produce a maculopapular rash on the trunk.
- b.** As the infection progresses, petechiae and hemorrhage of the mucous membranes develop, accompanied by blood in the urine and vomitus. Coagulopathy, microvascular damage, circulatory shock, and multiorgan failure characterize later stages of infection. Lassa fever (LF) and Rift Valley fever (RVF) viruses produce less serious hemorrhagic manifestation than SAHF, Crimean-Congo hemorrhagic fever (CCHF), and Ebola and Marburg hemorrhagic fever (E/MHF) infections. Severe necrosis of the liver and retinitis are unique to RVF. Depending on the pathogen, mortality rates range from low to very high (Ebola virus).
- c.** The mode of transmission of Ebola and Marburg viruses involves intimate contact with infected fluid and tissue.

## **4. Unknown virus**

- a.** The recognition of the initial signs of a viral epidemic within a population varies, depending on a number of factors, including mode of transmission, incubation period, and clinical presentation.
- b.** Viral shedding usually occurs before onset of symptoms and can continue through the course of the illness. Collection of specimens during early infection enhances the ability of the laboratory to recover or detect the virus.

- c. Transmission occurs either through direct contact with an infected individual (or animal), exposure to contaminated fomites, or through the bite of an insect vector. An epidemic propagated through person-to-person contact spreads more slowly through a population. Viral exposure through a common source, such as contaminated food, water, or aerosolized product, results in concurrent disease in a larger number of individuals.
- d. The laboratory must be alert to an unknown virus in a variety of specimens. Some viral infections disseminate from a primary infection site to a secondary location within the host. For example, smallpox is shed from the upper respiratory mucosa during the very early stages of infection, but is found in larger concentrations in the characteristic skin lesions that develop later. Differentiation of the unknown virus from other pathogens (Table 3) is important for infection control and rapid detection of an infection.

**TABLE 3. Diseases with symptoms compatible with smallpox or other viral bioterrorism agents**

Pre-exanthem	Early exanthem	Late exanthem
Influenza	Measles, rubella	Chickenpox
Sepsis	Varicella-zoster	Erythema multiforme
CNS infection	Misc. viral exanthem <sup>a</sup>	Stevens-Johnson syndrome
Appendicitis	Drug eruptions	Scabies
Pneumonia	Syphilis	Impetigo
Leukemia	Erythema multiforme	Drug eruptions
Enteric fever	Insect bites	Pemphigus
	Meningococemia <sup>b</sup>	Misc. viral exanthem <sup>a</sup>
		Meningococemia <sup>b</sup>

<sup>a</sup>A miscellaneous viral exanthem (skin rash) would include infections caused by herpes simplex virus, varicella-zoster virus, enteroviruses, or other poxviruses, such as the agents of vaccinia, molluscum contagiosum, or other poxviruses that can be passed from animals to humans such as monkeypox.

<sup>b</sup>Meningococemia could be confused with hemorrhagic smallpox.

## II. PROCEDURE

### A. General

1. If smallpox, filovirus, arenavirus, alphavirus, or Crimean-Congo hemorrhagic fever virus infection is suspected, contact public health officials before collection of specimens for diagnostic testing.
2. DO NOT inoculate specimens received for ruling out a potential viral agent of bioterrorism into cell culture. Immediately inform the appropriate personnel designated by the hospital's bioterrorism readiness protocol. Initiate direct communication with the local or state department of health.

## B. Precautions

1. Level A (Sentinel) laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the state health laboratory.
2. Obtaining a complete clinical history, including travel, animal, and vector exposure, provides valuable information for determining the etiologic agent of an infection. This information, in conjunction with the sequence of prodromal symptoms, allows rapid presumptive differentiation of these infections.
3. Follow standard precautions and use a safety hood that meets Biosafety Level 2 (BSL-2) specifications for processing any specimens, unless directed otherwise by the public health officials. BSL-2 recommendations include:
  - a. Wear gown and gloves while using the hood.
  - b. Before disposal, disinfect all material within the hood with a freshly prepared 1:10 dilution of bleach.
  - c. Air dry and perform fixation of smears within the safety hood. Perform fixation by placing slides in fixative in a container with a lid. Always replace the lid after addition of slides to minimize the presence of fumes within the hood. Keep the lid on the container at all times.
  - d. Perform centrifugation with polypropylene tubes with sealed lids. A safety shield should cover the rotor during centrifugation.

## C. Specimens

NOTE: DO NOT COLLECT specimens without authorization by public health officials. DO NOT USE viral transport medium unless specifically requested by public health officials.

1. **Smallpox.** Skin lesions (vesicle fluid, scrapings, crusts). Specific guidelines for collection of specimens for smallpox, and smallpox vaccine adverse events, are available at <http://www.bt.cdc.gov/labissues/index.asp>.
2. **Alphaviruses.** Respiratory secretions, blood, and cerebrospinal fluid, particularly during the first 2 to 3 days of infection; serum for serologic testing. Isolation of WEE is possible from autopsy brain tissue.
3. **VHF viruses.** Serum, heparinized plasma (not EDTA, citrate, or oxalate), whole blood, throat washings, tissue, and urine.
4. **Unknown virus.** Specimens sent for any type of viral or bacterial detection may harbor virus from a clinically unrecognized case.

## D. Specimen handling

1. Store specimens from patients with suspected virus in a tightly sealed protective container. Refrigerate while awaiting transport instructions. Retain tissue culture tubes suspected of containing the virus at 35°C.
2. Store paraffin blocks or formalin-fixed tissues at room temperature. Do not freeze.
3. Consult public health officials for transport instructions. Packaging of specimens must comply with regulations for shipping infectious substances.
4. Sentinel laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the state health laboratory.

### **E. Rejection criteria**

Appropriate criteria for specimen storage, preservation and transport were not followed. Save specimen under appropriate conditions until authorized to discard.

### **F. Recovery of virus in culture**

Unidentifiable patterns of cytopathic effect (CPE) could be caused by a potential agent of bioterrorism. Consult physician to see if the patient's clinical presentation is consistent with smallpox or a hemorrhagic viral infection. If the patient's history and/or disease is consistent, do not perform additional manipulations of the cell cultures. Notify the public health department and follow instructions to forward the culture and remaining specimens to the appropriate laboratory.

- 1. Smallpox.** The agent of smallpox (variola virus) will grow in cell lines used for herpesviruses. CPE is described as hypertrophic rounding of the cells in the monolayer.
- 2. Alphaviruses.** Cell lines that permit growth include MRC-5, A-549, Vero, LLCMK, and hamster kidney. CPE may only occur after passage.
- 3. VHF virus.** Filoviruses, arenaviruses, and SAHF viruses are cultivable in nonhuman primate and human cell lines, such as Vero and MRC-5.
- 4. CPE.** The CPE produced in culture often suggests a specific virus type. Laboratories then attempt to identify the virus by using a neutralization or antigen detection assay. Hemadsorption of specific types of human or animal erythrocytes to the cell monolayer determines the presence of a hemagglutinin-producing virus. None of the currently identified viral bioterrorism agents would produce monolayer hemadsorption.
- 5. Unidentifiable by standard methods.** If the virus is unidentifiable by standard methods, contact the patient's physician to confirm that the clinical history is consistent with a virus of bioterrorism. If the patient's history and/or disease is consistent with exposure to a viral bioterrorism agent, **DO NOT DESTROY** specimens or cultures. Contact public health officials for instructions. If the patient's history and/or disease is not consistent with a viral agent of bioterrorism, follow routine laboratory protocols.

### **G. Interpretation and reporting**

1. Document in the patient's report all findings for any specimen sent to the public health laboratory. Include the name of the public health laboratory.
2. Follow laboratory protocols for reporting the results to infection control practitioners and infectious disease physicians.

### **ACKNOWLEDGEMENT:**

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