Acute, Generalized Vesicular or Pustular Rash Illness Testing Protocol in the United States

Introduction

This protocol has been developed to guide the sequence and type of laboratory testing to be undertaken in situations involving specimens from patients with acute, generalized vesicular or pustular rash illness, or suspected smallpox vaccine (vaccinia virus) adverse event. This protocol may also be followed to test environmental specimens which may contain an Orthopoxvirus. The testing protocol includes four flowchart diagrams, each illustrating a different testing circumstance. The protocol has been designed for use alongside the Centers for Disease Control and Prevention's clinical assessment tool, Evaluating Patients for Smallpox: Acute, Generalized Vesicular or Pustular Rash Illness Protocol. The laboratory testing protocol is designed to address testing needs in a pre-event setting, when no poxvirus emergency has been detected or declared. In the event of a smallpox (variola virus) outbreak, critical updates will be announced by the Laboratory Response Network (LRN).

Chart 1 lists the major and minor criteria of smallpox used to categorize a patient's risk of smallpox – high, moderate, or low.

Chart 2 depicts the sequence of laboratory testing for specimens from patients with acute, generalized vesicular or pustular rash illness. The sequence and type of laboratory tests performed will depend on the outcome of the risk assessment using the clinical assessment tool, Evaluating Patients for Smallpox: Acute, Generalized Vesicular or Pustular Rash Illness Protocol (see Chart 1 for abstraction of the protocol). A two-armed testing algorithm is presented to ensure that testing of high-risk specimens is confined to laboratories with appropriate biosafety levels and expertise. This approach also reduces the time to receive test results.

Major points:

- High-risk specimens/cases require consultation with CDC prior to testing.
- Low-or moderate-risk specimens/cases should be worked-up for common causes of febrile exanthema.
- Due to differences in the thermocycling conditions used for the *Non-variola Orthopoxvirus* and *Orthopoxvirus* PCR assays, they cannot be performed simultaneously on the same instrument.

Chart 3 presents a testing algorithm that should be used when a smallpox vaccine adverse event is suspected.

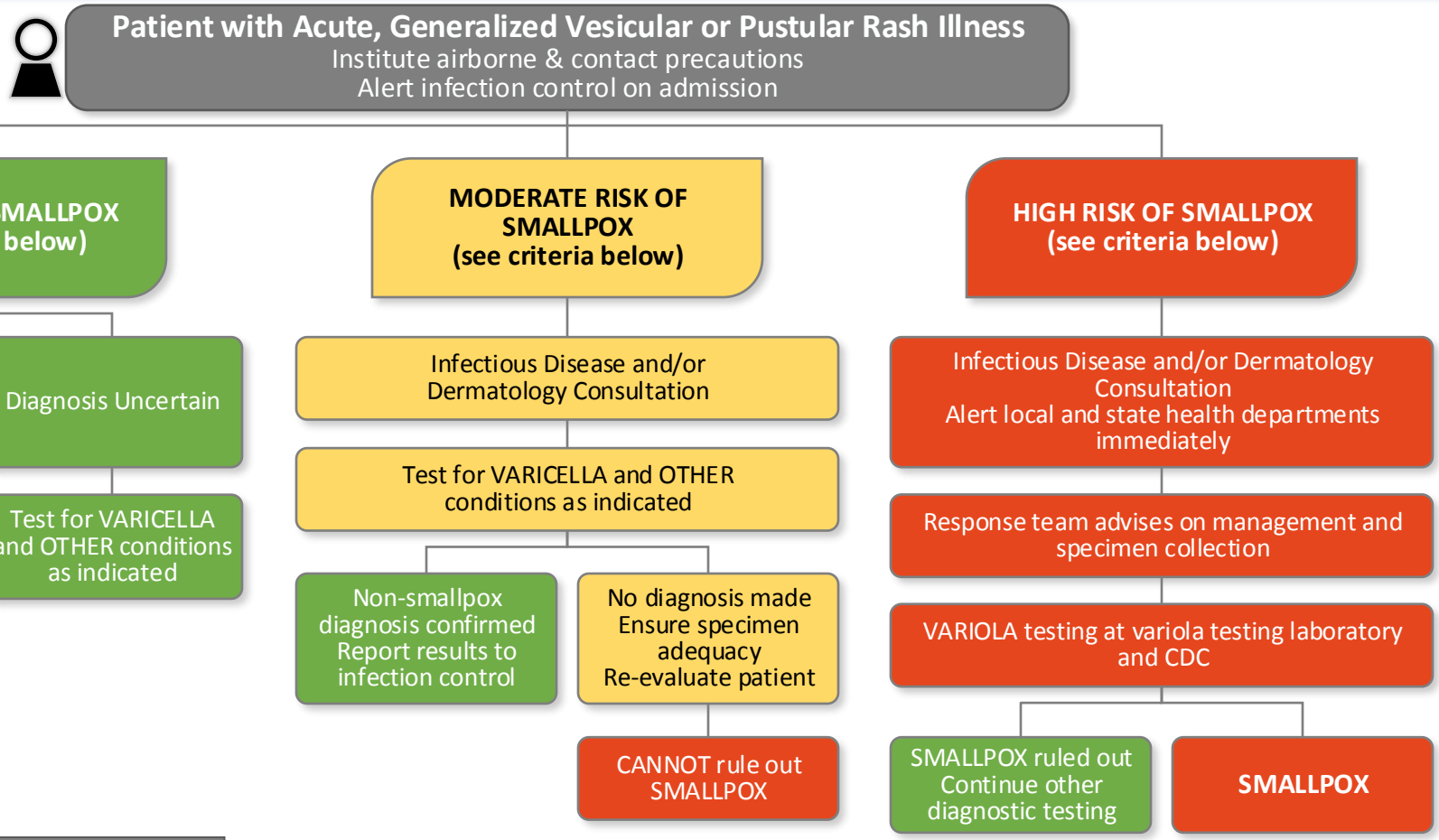
Chart 4 presents an Orthopoxvirus testing algorithm for environmental specimens. This testing algorithm should be used in conjunction with the LRN Multiple-Agent Screen.

When examining specimens from low- or moderate-risk cases it may be possible, though unlikely, that a positive result may be obtained using the Orthopoxvirus PCR assay and a negative result with Non-variola Orthopoxvirus PCR assay. This could signal a laboratory error, the presence of a previously uncharacterized Orthopoxvirus in the specimen, or that the patient has modified smallpox. Consultation with CDC should occur before running Variola virus specific PCR assays on specimens not initially determined to be high-risk.

The testing protocols are supported at the LRN reference laboratories. Details on the performance and interpretation of each assay are specified in each LRN procedure. Communication between laboratories and local or state epidemiologists is encouraged prior to laboratory testing, especially if high-risk specimens are involved. Refer to LRN policy regarding notification of test results.

CHART 1

Acute, Generalized Vesicular or Pustular Rash Illness Protocol



Risk of Smallpox
HIGH RISK OF SMALLPOX • Febrile prodrome **AND** • Classic smallpox lesions **AND** • Lesions in same stage of development
MODERATE RISK OF SMALLPOX • Febrile prodrome **AND** one other MAJOR smallpox criterion **OR** • Febrile prodrome **AND** ≥4 MINOR criteria
LOW RISK OF SMALLPOX • No febrile prodrome **OR** • Febrile prodrome **AND** <4 MINOR criteria

MAJOR Smallpox Criteria
• **Febrile prodrome:** Fever of ≥101°F, 1–4 days prior to rash onset with at least prostration, headache, backache, chills, vomiting or severe abdominal pain
• **Classic smallpox lesions:** Deep-seated, firm/hard, round well-circumscribed vesicles or pustules; lesions may umbilicate or become confluent
• **Lesions in same stage of development:** On any one part of the body all lesions in same stage of development

MINOR Smallpox Criteria
• Centrifugal distribution of lesions
• First lesions on the oral mucosal palate, face, or forearms
• Patient appears toxic or moribund
• Slow evolution of lesions from macule, to papule, to vesicle (1–2 days each stage)
• Lesions on the palms and soles

The poster, *Evaluating Patients for Smallpox: Acute, Generalized Vesicular or Pustular Rash Illness Protocol*, can be found on CDC's smallpox website.

Acute, Generalized Vesicular or Pustular Rash Illness Protocol

1. When a patient presents with an acute, generalized vesicular or pustular rash illness, institute airborne and contact precautions. Alert infection control on admission.
2. Determine the patient's risk of smallpox using the MAJOR and MINOR criteria.
    a. There are three MAJOR criteria:
        i. Febrile prodrome: Fever of ≥101°F, 1–4 days prior to rash onset with at least prostration, headache, backache, chills, vomiting or severe abdominal pain
        ii. Classic smallpox lesions: Deep-seated, firm/hard, round well-circumscribed vesicles or pustules; lesions may umbilicate or become confluent
        iii. Lesions in same stage of development: On any one part of the body all lesions in same stage of development
    b. There are five MINOR criteria:
        i. Centrifugal distribution of lesions
        ii. First lesions on the oral mucosalpalate, face, or forearms
        iii. Patient appears toxic or moribund
        iv. Slow evolution of lesions from macule, to papule, to vesicle (1-2 days each stage)
        v. Lesions on the palms and soles
    c. If the patient has no febrile prodrome OR has febrile prodrome AND <4 MINOR criteria, the patient's risk of smallpox is **low**.
        i. If the patient has a history and exam suggestive of varicella, varicella testing is optional, and risk of smallpox is **low**.
        ii. If the diagnosis is uncertain, test for varicella and other conditions as indicated. Risk of smallpox is **low**.
    d. If the patient has a febrile prodrome AND one other MAJOR smallpox criterion OR if the patient has a febrile prodrome AND ≥ 4 MINOR criteria, the patient's risk of smallpox is **moderate**.
        i. Obtain Infectious Disease and/or Dermatology consultation.
        ii. Test for Varicella and other conditions as indicated.
            1. If tests confirm a non-smallpox diagnosis, report results to infection control. The patient's risk of smallpox is low.
            2. If no diagnosis is made after tests are completed, ensure specimen adequacy and re-evaluate the patient. At this point, the patient's risk of smallpox is **moderate**.
            3. If re-evaluation does not reveal a diagnosis, you CANNOT rule out smallpox. Patient's risk of smallpox is **HIGH**.
    e. If the patient has a febrile prodrome AND classic smallpox lesions AND lesions in the same stage of development, the patient's risk of smallpox is **HIGH**.
        i. Obtain Infectious Disease and/or Dermatology consultation and alert local and state public health departments immediately.
        ii. The Response team from the local and/or state public health department will advise on management and specimen collection.
        iii. Perform variola testing at variola testing laboratory and at CDC
            1. If variola testing rules out smallpox, continue other diagnostic testing. Patient's risk of smallpox is low.
            2. If variola is positive, then smallpox is confirmed.

CHART 2 Laboratory Testing for Acute, Generalized Vesicular or Pustular Rash Illness

Patient with Acute, Generalized Vesicular or Pustular Rash Illness
Evaluated by Healthcare Practitioner, Infectious Disease or Dermatology Specialist

Take digital photos of the rash presentation for clinical consultation.

Consultation with local and/or state health department

LOW OR MODERATE RISK SPECIMENS (Green or Yellow Box)

Sentinel and/or LRN Reference Laboratory

Use BSL-2 facilities
- Perform diagnostic assay(s) appropriate for etiologic agent detection.
  - – DFA – Electron microscopy *(if available)*
  - – PCR* – Viral culture *(if appropriate)*

**Assays with the same cycling conditions can be run in parallel.*

If VARICELLA is suspected begin testing; consider using Tzanck smear.

Etiologic agent detection assay(s) other than *Orthopoxvirus*: **POSITIVE**

***Orthopoxvirus* ruled out**
No further testing needed unless clinically indicated.

Etiologic agent detection assay(s) other than *Orthopoxvirus*: **NEGATIVE**

Possible Orthopoxvirus
Perform the following:
- *Non-variola Orthopoxvirus* PCR
- *Orthopoxvirus* PCR

Non-variola Orthopoxvirus PCR: **NEGATIVE**
Orthopoxvirus PCR: **NEGATIVE**
[*Variola virus* PCR: **NEGATIVE**]

***Orthopoxvirus* ruled out**
- Re-evaluate the patient's condition and assess the need for dermatologic consultation and/or other diagnostic testing.
- Consider histologic testing for erythema multiforme, immune or drug reactions.

Non-variola Orthopoxvirus PCR: **POSITIVE**
Orthopoxvirus PCR: **POSITIVE**
[*Variola virus* PCR: **NEGATIVE**]

Possible Orthopoxvirus infection
(e.g., vaccinia, monkeypox, or cowpox virus)
- If needed, contact CDC to submit specimen(s) for confirmatory testing and species typing.

CDC Emergency Operations Center 770.488.7100
(Available 24/7)

HIGH RISK SPECIMENS (Red Box)

Contact CDC Emergency Operations Center BEFORE testing to discuss patient clinical history.
Emergency Operations Center: 770.488.7100 *(Available 24/7)*

Non-variola Testing Laboratory
Initiate chain-of-custody documentation at FBI direction.
Refer to variola testing laboratory.

Variola Testing Laboratory
Enhanced BSL-3 facilities with up-to-date smallpox vaccinated personnel required.

Simultaneously split specimens for testing at CDC

Electron microscopy at local facility AFTER BSL-3 preparation of grids

Rule out VARIOLA prior to other testing
Perform the following:
- *Variola virus* PCR
- *Orthopoxvirus* PCR
- *Non-variola Orthopoxvirus* PCR
- Electron microscopy *(if available)*

Non-variola Orthopoxvirus PCR: **NEGATIVE**
Orthopoxvirus PCR: **POSITIVE**
Variola virus PCR: **POSITIVE**

Possible SMALLPOX infection†
- Contact CDC immediately PRIOR to release of results.

CDC Emergency Operations Center 770.488.7100
(Available 24/7)
†Could also represent differential sensitivities of the assays.

NOTE: If patient symptoms progress to more closely resemble smallpox, refer all specimens to a Variola Testing Laboratory and CDC. Sequester all viral cultures and specimens. Contact PHL for transport of specimens.

Laboratory Testing for Acute, Generalized Vesicular or Pustular Rash Illness

1. A patient presents with an acute, generalized vesicular or pustular rash illness and is evaluated by a healthcare practitioner, infectious disease, or dermatology specialist.
    a. Take digital photos of the rash presentation for clinical consultation.
    b. Obtain consultation with local and/or state public health department.
2. If patient evaluation determines the patient has a low or moderate risk of smallpox, the specimens for testing will also be low or moderate risk and will be tested by a sentinel and/or an LRN Reference Laboratory.
    a. Use BSL-2 facilities to perform diagnostic assay(s) appropriate for etiologic agent detection. Options are:
        i. DFA
        ii. PCR (assays with the same cycling conditions can run in parallel)
        iii. Electron microscopy (if available)
        iv. Viral culture (if appropriate)
    b. If varicella is suspected begin testing; consider using Tzanck smear.
    c. If etiologic agent detection assay(s) other than *Orthopoxvirus* are positive:
        i. *Orthopoxvirus* is ruled out. No further testing is needed unless clinically indicated. The patient has a low risk of smallpox.
    d. If etiologic agent detection assay(s) other than *Orthopoxvirus* are negative:
        i. *Orthopoxvirus* is possible.
        ii. Perform the following tests:
            1. *Non-variola Orthopoxvirus* PCR
            2. *Orthopoxvirus* PCR
        iii. If the *Non-variola Orthopoxvirus* PCR and *Orthopoxvirus* PCR are both negative, *Orthopoxvirus* is ruled out.
            1. Re-evaluate the patient's condition and assess the need for dermatologic consultation and/or other diagnostic testing.
            2. Consider histologic testing for erythema multiforme, immune or drug reactions.
        iv. If the *Non-variola Orthopoxvirus* PCR and the *Orthopoxvirus* PCR are both positive:
            1. *Orthopoxvirus* infection other than variola virus (e.g., vaccinia, monkeypox, or cowpox virus) is possible.
            2. If needed, contact CDC to submit specimen(s) for confirmatory testing and species typing. The CDC Emergency Operations Center is available 24/7 at 770-488-7100.
3. If patient evaluation determines the patient has a **HIGH** risk of smallpox:
    a. Contact CDC Emergency Operations Center at 770-488-7100 before testing to discuss patient clinical history.
    b. If specimens are at a non-variola testing laboratory:
        i. Initiate chain-of-custody at FBI direction. Refer to variola testing laboratory.
    c. If specimens are at a variola testing laboratory:
        i. Use enhanced BSL-3 facilities. It is required that testing personnel be up-to-date with their smallpox vaccination.
        ii. Simultaneously split specimens for testing at CDC.
    d. Rule out variola prior to other testing. Perform the following:
        i. *Variola virus* PCR
        ii. *Orthopoxvirus* PCR
        iii. *Non-variola Orthopoxvirus* PCR

iv. Electron microscopy (if available). Perform electron microscopy at local facility AFTER BSL-3 preparation of grids.

e. If the *Non-orthopoxvirus* PCR is negative, the *Orthopoxvirus* PCR is negative, and *Variola virus* PCR is negative:
   i. *Orthopoxvirus* is ruled out.
   ii. Re-evaluate the patient's condition and assess the need for dermatologic consultation and/or other diagnostic testing.
   iii. Consider histologic testing for erythema multiforme, immune or drug reactions.

f. If the *Non-orthopoxvirus* PCR is positive, the *Orthopoxvirus* PCR is positive, and *Variola virus* PCR is negative:
   i. *Orthopoxvirus* infection other than variola virus (e.g., vaccinia, monkeypox, or cowpox virus) is possible.
   ii. If needed, contact CDC to submit specimen(s) for confirmatory testing and species typing. The CDC Emergency Operations Center is available 24/7 at 770-488-7100.

g. If the *Non-orthopoxvirus* PCR is negative, the *Orthopoxvirus* PCR is positive, and *Variola virus* PCR is negative:
   i. Smallpox infection is possible (though this could also represent different sensitivities of the assays)
   ii. Contact CDC immediately PRIOR to release of results. Call CDC Emergency Operations Center at 770-488-7100 (available 24/7)

4. NOTE: If patient symptoms progress to more closely resemble smallpox, refer all specimens to a Variola Testing Laboratory and CDC. Sequester all viral cultures and specimens. Contact PHL for transport of specimens.

CHART 3 Laboratory Testing for Suspect Smallpox Vaccine Adverse Event (Vaccinia)

Patient with Suspect Smallpox Vaccine Rash Illness
Evaluated by Healthcare Practitioner, Infectious Disease or Dermatology Specialist

Take digital photos of the rash presentation for clinical consultation.

Consultation with local and/or state health department

LRN Reference Laboratories
WITH *Orthopoxvirus* PCR capacity
All other laboratories refer

Use BSL-2 facilities
Perform the following:
- *Non-variola Orthopoxvirus* PCR
- *Orthopoxvirus* PCR
- Electron microscopy *(if available)*

Non-variola Orthopoxvirus PCR: **NEGATIVE** *Orthopoxvirus* PCR: **NEGATIVE**	*Non-variola Orthopoxvirus* PCR: **POSITIVE** *Orthopoxvirus* PCR: **POSITIVE**	*Non-variola Orthopoxvirus* PCR: **NEGATIVE** *Orthopoxvirus* PCR: **POSITIVE**
Orthopoxvirus ruled out	***Possible Orthopoxvirus infection*** ***(e.g., vaccinia, monkeypox, or cowpox virus)***	***Possible SMALLPOX infection†***
• Re-evaluate the patient's condition and assess the need for dermatologic consultation and/or other diagnostic testing. • Consider histologic testing for erythema multiforme, immune or drug reactions.	• If needed, contact CDC to submit specimen(s) for confirmatory testing and species typing. CDC Emergency Operations Center 770.488.7100 *(Available 24/7)*	• Contact CDC immediately PRIOR to release of results. CDC Emergency Operations Center 770.488.7100 *(Available 24/7)* *†Could also represent differential sensitivities of the assays.*

Laboratory Testing for Suspect Smallpox Vaccine Adverse Event (Vaccinia)

1. A patient presents with suspect smallpox vaccine rash illness and is evaluated by healthcare practitioner, infectious disease or dermatology specialist
    a. Take digital photos of the rash presentation for clinical consultation
2. Consult with local and/or state health department
3. Laboratory testing occurs in a LRN Reference Laboratory with *Orthopoxvirus* PCR capacity. All other laboratories refer.
4. LRN Reference Laboratory with *Orthopoxvirus* PCR capabilities must use BSL-2 facilities to perform the following tests:
    a. *Non-variola Orthopoxvirus* PCR
    b. *Orthopoxvirus* PCR
    c. Electron microscopy *(if available)*
5. If the *Non-variola Orthopoxvirus* PCR is negative and the *Orthopoxvirus* PCR is negative, *Orthopoxvirus* is ruled out. The risk is low.
    a. Re-evaluate the patient's condition and assess the need for dermatologic consultation and/or other diagnostic testing.
    b. Consider histologic testing for erythema multiforme, immune or drug reactions.
6. If the *Non-variola Orthopoxvirus* PCR is positive and the *Orthopoxvirus* PCR is positive, the patient has a possible *Orthopoxvirus* infection other than variola virus (e.g., vaccinia, monkeypox, or cowpox virus).
    a. If needed, contact CDC to submit specimen(s) for confirmatory testing and species typing. The CDC Emergency Operations Center is available 24/7 at 770-488-7100.
7. If the *Non-variola Orthopoxvirus* PCR is negative and the *Orthopoxvirus* PCR is positive, the patient has a possible smallpox infection. The results could also represent differential sensitives of the assays.
    a. Contact CDC immediately PRIOR to release of results. The CDC Emergency Operations Center is available 24/7 at 770-488-7100.

CHART 4

Laboratory Testing for Environmental Samples

Environmental Samples
Law Enforcement Credible Threat Assessment

Initiate chain of custody documentation

LRN Reference Laboratories
WITH *Orthopoxvirus* PCR capacity
All other laboratories refer

Use BSL-3 facilities

- Perform PCR screening panel for environmental biothreat agent DNA detection.

Orthopoxvirus result from PCR screening panel: **NEGATIVE**

Orthopoxvirus ruled out

- Assess need for further testing with law enforcement.

Orthopoxvirus result from PCR screening panel: **POSITIVE**

Possible Orthopoxvirus

- Perform *Orthopoxvirus* PCR assay to verify result from PCR screening panel.

Orthopoxvirus PCR: **NEGATIVE**

Orthopoxvirus CANNOT be ruled out

- Investigate the cause of discrepant PCR results between runs; implement corrective actions.
- If needed, contact CDC to discuss PCR results and the need for additional testing.

CDC Emergency Operations Center 770.488.7100
(Available 24/7)

Orthopoxvirus PCR: **POSITIVE**

Possible Orthopoxvirus

- Contact CDC to submit specimen(s) for confirmatory testing and species typing.

CDC Emergency Operations Center 770.488.7100
(Available 24/7)

Laboratory Testing for Environmental Samples

1. Upon receipt of environmental samples, which law enforcement requires a credible threat assessment, initiate chain-of-custody documentation.
2. Laboratory testing occurs in a LRN Reference Laboratory with *Orthopoxvirus* PCR capacity. All other laboratories refer.
3. LRN Reference Laboratory with *Orthopoxvirus* PCR capabilities must use BSL-3 facilities to perform PCR screening panel for environmental biothreat agent DNA detection.
4. If the *Orthopoxvirus* result from PCR screening panel is negative, *Orthopoxvirus* is ruled out.
    a. Assess need for further testing with law enforcement.
5. If the *Orthopoxvirus* result from PCR screening panel is positive, *Orthopoxvirus* is possible.
    a. Perform *Orthopoxvirus* PCR assay to verify result from PCR screening panel.
        i. If *Orthopoxvirus* PCR is negative, *Orthopoxvirus* cannot be ruled out:
            1. Investigate the cause of discrepant PCR results between runs; implement corrective actions.
            2. If needed, contact CDC to discuss PCR results and the need for additional testing. The CDC Emergency Operations Center is available 24/7 at 770-488-7100.
        ii. If *Orthopoxvirus* PCR is positive, *Orthopoxvirus* is possible:
            1. Contact CDC to submit specimen(s) for confirmatory testing and species typing. The CDC Emergency Operations Center is available 24/7 at 770-488-7100.