Introduction
This protocol has been developed to illustrate the types of laboratory testing to be undertaken in different situations involving patients with acute, generalized vesicular or pustular rash illness. The protocol is composed of four charts, each illustrating a different set of symptoms or circumstances. It has been designed to correlate with the CDC Poster: “Evaluating Patients for Smallpox” (http://www.bt.cdc.gov/agent/smallpox/diagnosis/evalposter.asp). This is a pre-event algorithm, designed to address testing needs when no poxvirus emergency has been detected or declared. Changes to this algorithm may be required in an event. Any updates to the algorithm will be announced by the Laboratory Response Network.

Chart 1 lists the symptoms associated with acute, generalized vesicular or pustular rash illness and categorizes the risk of smallpox according to the patient’s signs and symptoms.

Chart 2 presents a flow chart for laboratory testing of specimens from patients presenting with acute, generalized vesicular or pustular rash illness, following the assessment per the “Acute, Generalized Vesicular or Pustular Rash Illness” protocol (see above and chart 1 for abstraction of the protocol). A two-armed algorithm is presented to reduce the time to receive results and to ensure that testing of high-risk specimens is confined to laboratories with appropriate biosafety levels and expertise. The two arms of the testing algorithm are for 1) specimens from individuals with low-and moderate-risk symptoms and 2) specimens from individuals with high-risk symptoms.

Major points:
   a) high-risk specimens/cases require consultation with CDC
   b) low-or moderate-risk specimens/cases should be worked-up for more common causes of febrile exanthema. Due to the differences in run temperature between the Non-variola Orthopoxvirus PCR and the Orthopoxvirus PCR, the two assays cannot be performed simultaneously. If Orthopoxvirus is suspected, then the Non-variola Orthopoxvirus PCR permits the laboratory to identify an Orthopoxvirus and reassure that the specimen does not contain variola. The Orthopoxvirus generic PCR can be performed subsequently to provide additional confidence in the result.
   c) in the absence of endemic smallpox disease, the indiscriminate use of variola tests will lead to false positives.

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

Chart 4 presents an Orthopoxvirus testing algorithm for environmental samples.

The testing protocols are supported at Laboratory Response Network (LRN) reference laboratories. Details for performance and interpretation of each assay are specified in each LRN procedure.

Details on specimen collection can be found at the following websites:
Vaccine adverse events: www.bt.cdc.gov/agent/smallpox/vaccination/vaccinia-specimen-collection.asp
Monkeypox: www.cdc.gov/ncidod/monkeypox/diagspecimens.htm
ACUTE, GENERALIZED VESICULAR OR PUSTULAR RASH ILLNESS PROTOCOL

Patient with Acute, Generalized Vesicular or Pustular Rash Illness

Institute Airborne & Contact Precautions
Alert Infection Control on Admission

Low Risk of Smallpox
(see criteria below)

- History and Exam
  Highly Suggestive of Varicella

- Diagnosis Uncertain

- Varicella Testing Optional

Moderate Risk of Smallpox
(see criteria below)

- ID and/or Derm Consultation
  VZV +/- Other Lab Testing as Indicated

- No Diagnosis Made
  Ensure Adequacy of Specimen
  ID or Derm Consultant
  Re-evaluate Patient

- Cannot R/O Smallpox

High Risk of Smallpox
(see criteria below)

- ID and/or Derm Consultation
  Local and State Health Depts

- Appropriate Treatment for Varicella/Other Conditions
  as Clinically Indicated

- Response Team Advises on Management and Specimen Collection

- Testing at CDC

Risk of Smallpox

**High Risk of Smallpox**
1. Febrile prodrome **AND**
2. Classic smallpox lesion **AND**
3. Lesions in same stage of development in any area of the body

**Moderate Risk of Smallpox**
1. Febrile prodome **AND** one other MAJOR smallpox criterion **OR**
2. Febrile prodome **AND** ≥ 4 MINOR smallpox criteria

**Low Risk of Smallpox**
1. Febrile prodome **OR**
2. Febrile prodome **AND** <4 MINOR smallpox criteria

**Major Smallpox Criteria:**
- Febrile prodrome ≥101°F, 1-4 days prior to rash onset
- with headache, back ache, or abdominal pain
- Firm, deep-seated, well circumscribed vesicles/pustules
- Lesions in the same stage of development in any area of the body

**Minor Smallpox Criteria:**
- Centrifugal distribution of lesions
- First lesions in the pharynx, oral mucosa, face, or forearms
- Slow evolution of rash
  - 1-2 days each stage: macule, papule, vesicle
- Lesions on the palms and soles
LABORATORY TESTING FOR ACUTE, GENERALIZED VESICULAR OR PUSTULAR RASH ILLNESS IN THE UNITED STATES

**Low and Moderate Risk Specimens**
(Green and Yellow Boxes)
- Sentinel Laboratories and/or LRN Reference Laboratories

**High-Risk Specimens**
(Red Box)
- Immediately refer to Variola Testing Laboratory
  - Initiate Chain-of-Custody documentation at FBI direction

**Use BSL-2 facilities**
- DFA: VZV and HSV
- PCR: VZV, HSV, Enterovirus (where available)
- EM: (where available)
- Viral culture: as appropriate
  - Consider biopsy for erythema multiforme

**If VZV diagnosis is questionable begin lab testing**

**Consider:**
- Tzanck smear (herpesviruses)
- EM (if available)

**Non-virion Orthopoxvirus PCR: POS**
Vaccine-related adverse event or possible monkeypox.
**Contact CDC Emergency Operation Center (EOC) 770-488-7100**
For verification of the result, the Orthopoxvirus PCR may be used.

**Variola Testing Laboratories Enhanced BSL-3 required**
- Variola PCR
- Orthopoxvirus PCR
- Non-virion Orthopoxvirus PCR
- EM (if available)

**All orthopox tests Neg:**
Perform the following:
- DFA: VZV and HSV
- PCR: VZV, HSV, Enterovirus
- Viral culture and other diagnostic tests as clinically indicated

**If VZV, HSV, Enterovirus or other non-Orthopoxvirus POS**
Non-Orthopoxvirus Diagnosis
- No further testing (unless clinically indicated)
- Re-evaluate patient condition and assess need for dermatologic and histologic testing including tests for erythema multiforme; consider biopsy.
- For additional confirmation that Orthopoxvirus is not present in the specimen, the Orthopoxvirus PCR may be used.

**If all tests are NEG:**
- Variola & Orthopoxvirus PCR both POSITIVE
  - Highly suggestive of SMALLPOX
  - CALL CDC immediately prior to release of results
- Variola PCR POS & Orthopoxvirus PCR NEG=
  - Vaccine-related adverse event or monkeypox.
  - **Contact CDC Emergency Operations Center (EOC) 770-488-7100**

**If patient symptoms progress to more closely resemble smallpox, refer all specimens to CDC and/or LRN labs with Variola PCR testing capability. Sequester all viral cultures and specimens. Contact PHL for transport of specimens.**

**Consultation with PHL and CDC**
- Rule out variola, prior to other testing, at laboratory with specific variola test capacity. LRN-designated Variola Testing Laboratories and CDC will have this capacity. CDC will test split sample simultaneously.
- **Results must not be released without CDC confirmation.**
  - Once reliable performance of assays in surge labs is demonstrated (post-event) CDC confirmation may be discontinued.
**Laboratory Testing for Suspected Smallpox Vaccine (Vaccinia) Adverse Events or Monkeypox in the United States**

**Patient with Suspected Vaccine-Related Adverse Event or Monkeypox**

- **Acceptable specimens:**
  - Vesicular "touch prep"  
  - Vesicle roof  
  - Vesicular swab  
  - Ocular swab or impression slide  
  - Biopsy specimens

**LRN Labs (with PCR Capability)**
- Non-variola Orthopoxvirus PCR
- Orthopoxvirus PCR
- EM – (if available)

**Test Results**

- **Non-variola orthopoxvirus PCR:**
  - **POS**
  - EM (optional): **POS** for poxvirus

- **Non-variola orthopoxvirus PCR:**
  - **NEG**
  - EM (optional): **NEG** for poxvirus

- **Non-variola orthopoxvirus PCR:**
  - **NEG**
  - EM: **POS or NEG** for poxvirus

**Vaccine-related adverse event or monkeypox**
- Evaluate exposure history and contact CDC to submit specimen for confirmatory tests.
- Contact CDC Emergency Operations Center (EOC) 770-488-7100

**Note:**
- A non-variola orthopoxvirus PCR POS and orthopoxvirus PCR NEG should not be generated.
- If that occurs, consult CDC Emergency Operations Center (EOC) 770-488-7100

**Orthopoxvirus identified – possible variola**
- Perform:
  - DFA: VZV, HSV
  - PCR: VZV, HSV, Enterovirus
- Viral culture and other diagnostic tests as clinically indicated
- Refer immediately to CDC for confirmatory testing: Emergency Operations Center (EOC) 770-488-7100

**Note:** Could also represent differential sensitivities of the assays

**Additional Recommendations:**
- Take digital photos of clinical presentations for electronic consultations.

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**Includes any non-variola orthopoxvirus, such as vaccinia, monkeypox and cowpox**
Initiate Chain-of-Custody documentation

Environmental Samples Law Enforcement Credible Threat Assessment
(Explosives, radiation, hazardous chemicals and toxins ruled out)

Sentinel Laboratory Refer only

LRN Reference Laboratory
- Orthopox PCR
- EM (if available)

Test Results

Orthopoxvirus PCR: **NEG**
EM: **NEG** for poxvirus

Orthopoxvirus ruled out.
Assess need for further testing with law enforcement. Report negative results to other groups investigating specimens.

Orthopoxvirus PCR: **POS**
EM: **POS** or **NEG** for poxvirus

Orthopoxvirus material identified – possible variola*
Refer immediately to CDC for confirmatory testing
Emergency Operations Center (EOC)
770-488-7100