

## **Guidelines for the Investigation of Potential Poxvirus and other Febrile Vesicular Rash Illnesses**

### **I. General**

Public health laboratories have developed the capacity to investigate cases of poxvirus and other febrile vesicular rash illnesses. Those agents that need the most consideration in the differential diagnosis of poxvirus and look alike illnesses are chickenpox, *Herpes simplex* virus, monkey pox, smallpox and post smallpox vaccination illness. Local laboratories SHOULD NOT attempt to investigate cases of suspected smallpox infection. This virus is a biosafety level 4 (BSL-4) agent. Validated laboratory diagnosis of *Variola major* and *V. minor* (agents for smallpox) is not available outside public health laboratories. Furthermore, state laboratories are working closely with the Centers for Disease Control and Prevention (CDC), which has the capability to evaluate diseases caused by bioengineered agents.

In the United States, suspected cases of smallpox must immediately be reported to the state or territorial health department. These cases must be immediately reviewed with state and local health officials to collate all pertinent information, initiate the emergency response protocol, and ensure that appropriate specimens are collected. This document was created to distribute guidelines on collection and shipment of related specimens in a suspected case of poxvirus, investigation of post-vaccinia vaccination adverse affects, or other febrile vesicular rash illnesses. You are required to take the following actions in such situations:

- Immediately contact the Michigan Department of Community Health (MDCH) laboratory director at 517-335-8063 and the MDCH epidemiologist/health officer at 517-335-8165 during normal business hours. After hours call 517-335-9030.
- Be prepared to provide pertinent patient information and emergency 24/7 contact information of the laboratory, attending, and consulting, or ED physicians.
- Pursuant to a potential criminal investigation, original specimens should be biologically contained (refrigeration may be necessary) and appropriately secured.
- Digital photographs of clinical presentation may be requested for electronic consultation.

After specimens are collected and submitted to MDCH:

- *Varicella zoster* virus (VZV) and *Herpes simplex* virus (HSV) testing will be done initially on specimens submitted to the MDCH laboratory. If this testing is negative and a consultation with the submitting physician indicates the patient's condition is consistent with poxvirus illness, poxvirus testing will be initiated at the MDCH laboratory.
- When this initial poxvirus testing is negative but there is a strong suspicion for a case of smallpox, MDCH will notify CDC for further smallpox testing.
- Administration of smallpox vaccination, and need for Vaccinia Immune Globulin (VIG) should be determined in coordination with MDCH. In the event of a large outbreak of confirmed smallpox, other laboratories with smallpox diagnostic capabilities may be used for diagnostic surge capacity. These laboratories will be designated by MDCH and instructions for sending specimens to these laboratories will be issued at the time of their designation.

### **II. Safety**

Recently immunized personnel (successfully vaccinated against smallpox within the past three years) wearing appropriate personal protective equipment are the best personnel to be involved in specimen collection for suspected high or moderate risk cases of smallpox. Appropriate barrier protection consists of gloves, disposable lab coat, and shoe covers; 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, or those with questionable vaccine status, 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.

(<http://emergency.cdc.gov/agent/smallpox/vaccination/contraindications-clinic.asp>) Fit-tested N-95 masks should be worn by unvaccinated individuals when collecting specimens from suspect patients. For collection of low risk specimens, including those associated with vaccination or associated with a known non-smallpox poxvirus outbreak, recently vaccinated personnel are not required. See the algorithm on the CDC web page for patient evaluation criteria.

(<http://emergency.cdc.gov/agents/smallpox/diagnosis/pdf/poxalgorithm11-14-07.pdf>)

- A. All procedures for processing and packaging of potentially infectious material should be performed utilizing BSL-2 or, if available, BSL-3 practices.
- B. While working with specimens, laboratory personnel should avoid any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, inserting contact lenses, or applying cosmetics.
- C. After removing gloves, personnel should thoroughly wash their hands with soap containing Lysol or soaps such as Hibiclens before leaving the laboratory. Areas of the skin known or suspected to have come in contact with the specimen should be washed with soap and should be decontaminated with a 10% solution of 0.5% sodium hypochlorite (bleach); allow at least a 1-minute contact time.
- D. After specimen collection is completed, all protective materials worn by the specimen collector (e.g., gloves, mask, lab coat, shoe covers) and all non-reusable sample collection materials and equipment (e.g., needles, tubes, swabs) must be placed in biohazard bags or sharps containers (where appropriate) and autoclaved or incinerated before disposal.
- E. In addition to these precautions, follow your facility's protocols and guidance with regard to appropriate infection control activities.

### III. Specimen Collection Supply List

The following materials will be required for specimen collection from each patient:

#### **Personal Protective Equipment (Not provided by MDCH)**

- Disposable protective latex, nitrile or vinyl gloves (sterile gloves not required)
- Disposable protective lab coats
- N-95 masks or higher rated, properly fitted HEPA – filtered respirators
- Protective eyewear or facemask
- Shoe covers

#### **MDCH Unit # 20 Vaccinia/Variola/Pox Virus Unit**

- Ten sterile dry Dacron swabs
- Ten sterile screw-capped 2 ml vials
- One 10 ml plastic marble - topped tube, serum separator
- MDCH test requisition
- UN 6.2 infectious substance shipping container
- Ice substitute
- Packaging and shipping materials

#### **Equipment/Materials Which May Be Needed but Are NOT Provided in MDCH Collection Unit**

- Biohazard plastic disposable bags
- One disposable scalpel with No. 10 blade
- Several sterile 26 gauge needles
- One 3.5 or 4 mm punch biopsy kit
- One vacutainer holder
- Two vacutainer needles (20 x 1 ½ in.)
- Parafilm
- Formalin (if available)
- 10% solution of 0.5% sodium hypochlorite (bleach)
- Clean plastic or glass microscope slides (if available)
- Slide holders (if including microscope slides)
- Alcohol wipes

#### **IV. Specimen Collection Procedure**

##### **Acceptable Specimens**

- Vesicular material (vesicle roof, vesicle smear from lesion still at blister stage)
- Lesion swab
- Lesion scab (from late stage lesion)
- Biopsy tissue
- Ocular impression (external surfaces, i.e., lesions, conjunctivae or eyelids, are areas deemed suspicious for viral infection)
- Serum

##### **Information required on MDCH Test Requisition Form:**

- Patient name
- Date and time of specimen collection
- Source and quantity of specimen
- Date of birth of patient
- Patient gender, race, ancestry and city of residence.
- Name and phone number of agency submitter and physician(s)
- Hospital identification number, if the patient is hospitalized.
- Information on patient condition and development and description of rash.

**Put on the protective equipment described above in Safety (Section II) for the procedures described below.** After specimen collection is complete, all personal protective materials and sample collection materials must be placed in biohazard bags or sharps containers (where appropriate) and autoclaved or incinerated prior to disposal. Refer to decontamination guidelines VII below.

**NOTE: Because the test algorithm includes HSV and VZV testing to rule -in these viral infections, it is preferable to collect multiple specimens from multiple lesions/sites. The greatest opportunity to recover virus occurs when sampling multiple specimens, e.g., scab, roof, swab, and touch prep**

Do not use glass vials if possible. Use plastic vials or bottles as the primary container for ALL specimens.

##### **A. Vesicular Material**

1. Sanitize skin with alcohol wipe and allow to completely dry.
2. Open and remove the top of the lesion using a sterile scalpel and/or 26-gauge needle.

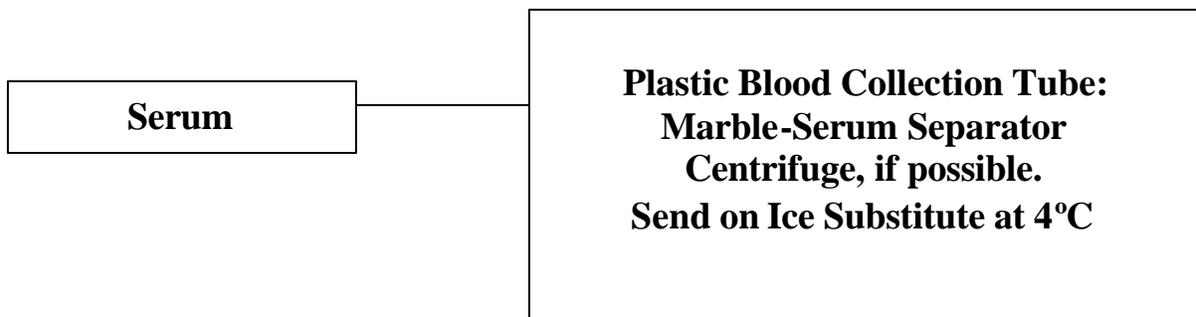
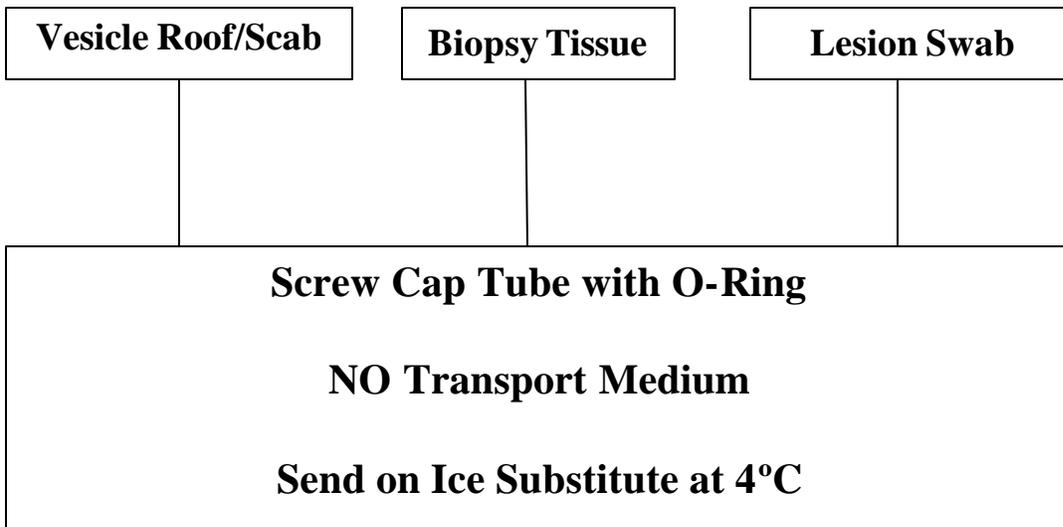
3. Place the vesicle skin “roof” in a dry, sterile 1.5-2.0 ml screw-capped plastic vial with O-ring. Cap vial to maintain relative sterility, and keep vial in as cool an environment as reasonably possible to preserve virus viability. DO NOT add transport medium to the vial.
  4. Scrape the base of the blister with the blunt edge of the scalpel or a wooden applicator and smear the scrapings onto a microscope slide.
  5. Touch a microscope slide multiple times to the open lesion.
  6. Allow slides to air dry for about 10 minutes.
  7. Repeat steps 1-6 with 2 or more lesions , if possible .
- B. Lesion Swab
1. Sanitize skin with alcohol wipe and allow to completely dry.
  2. Open and remove the top of the lesion using a sterile scalpel and/or 26-gauge needle.
  3. Swab the base of the lesion with a Dacron swab, place in a screw-capped plastic vial, break off swab handle and screw on cap. DO NOT add transport medium to the vial.
  4. Repeat steps 1-4 with 2 or more lesions , if possible .
- C. Lesion Scab
1. Remove scabs from 2-4 lesions , if possible, using a sterile scalpel and/or 26-gauge needle.
  2. Place scabs in a sterile 1.5-2.0 ml screw-capped plastic vial with O-ring.
- D. Biopsy Tissue (if possible, obtain at least 2 separate lesions)
1. Use a 3.5-4.0 mm punch biopsy device to sample an entire lesion.
  2. If possible, bisect the biopsied material, using sterile scissors or scalpel.
  3. Place half the biopsied material in formalin.
  4. Place the other half of the biopsied material in a sterile 1.5-2.0 ml screw-capped plastic vial with O-ring. DO NOT add transport medium.
  5. Repeat with at least one more lesion.
- OR
1. Biopsy two lesions.
  2. Place 1 biopsy in formalin.
  3. Place the second biopsy in a sterile 1.5-2.0 ml screw-capped plastic vial with O-ring. DO NOT add transport medium.
- E. Ocular Impression Smears
1. Ocular impressions should only be collected by an ophthalmologist.
  2. Touch a microscope slide to the ocular site. Prepare 2-3 slides.
  3. Allow slides to air dry for approximately 10 minutes.
  4. Label the slides and place them into slide holder(s). Wrap slide holder with parafilm.
- F. Serum
1. Draw 10 cc of blood into a plastic serum separator tube (marble-topped or gold-topped).
  2. If possible, centrifuge specimen to separate serum from blood clot. DO NOT OPEN TUBE. Apply parafilm to the cap. Testing requires at least 1 ml of serum.
  3. Discard needle/syringe in biohazard sharps container. Autoclave sharps container immediately.

## V. Specimen Labeling and Storage

- Label each specimen with patient name and identifying number.
- If multiple specimens are collected, indicate specific source of specimen (e.g., roof, swab, scab, serum, biopsy).

- Tighten caps securely on all vials or tubes and apply parafilm to seal the caps. Place slides in appropriate, labeled containers. Wrap slide holder(s) with parafilm to prevent accidental opening.
- Store specimens at 2-8°C and transport to MDCH lab within 24 hours.

## Specimen Collection for Potential Poxvirus and other Febrile Vesicular Rash Illnesses



### NOTE:

- Put on personal protective equipment (PPE) before collecting specimens.
- For detailed procedures for each specimen please refer to page No. 3 in the guidelines.

## VI. Packaging and Transport of Specimens

- These specimens require special handling. Within 24 hours of collection, package and ship on ice substitute at 4 degrees C. Please follow the packaging instructions described below to properly prepare the specimens for shipment. **Contact MDCH at 517-335-8063 during normal business hours (after hours call 517-335-9030) to make arrangements for emergency transportation of these specimens** or if any questions arise concerning safe transport of infectious substances.
- Each patient's specimens must be packaged separately from other patient specimens to avoid possible cross-contamination.
  - Complete the "Vaccinia/Variola/Pox Virus Requisition" form. Place the completed requisition in the plastic bag provided to protect from moisture.
  - Complete the Dangerous Goods form, if indicated.
  - Using the packaging materials provided in Unit #20, Vaccinia/Variola/Pox Virus Unit, prepare specimens for transport. Place properly labeled specimen vials, wrapped in absorbent material provided, into the aluminum screw-capped can and secure cap with tape. Place aluminum can and test requisition into the cardboard shipping unit canister; seal the lid with tape and place into the UN 6.2 corrugated packaging.
  - Complete and apply the appropriate shipping labels provided to the outside of the styrofoam lined overpack box. Add the frozen ice substitute refrigerants to the overpack box and seal with tape. If indicated, attach the completed Dangerous Goods form to the outside of the overpack.
- Final instructions regarding packaging and transportation will be given at time of consultation.

## VII. Decontamination Guidelines

Only vaccinated personnel or personnel without contraindication to vaccination should perform decontamination procedures. (<http://emergency.cdc.gov/agent/smallpox/vaccination/contraindications-clinic.asp>) Protective clothing including disposable lab coats, gloves, shoe covers, caps, and masks must be worn. All personnel should adhere to proper hand hygiene practices as per current recommendations.

There are no disinfectant products registered by the U.S. Environmental Protection Agency (EPA) specifically for the inactivation of variola virus on surfaces, nor have any products been evaluated for this purpose using this specific virus. It has been established, however, that viruses with biophysical and biochemical properties similar to those of variola virus (e.g., vaccinia virus) are readily inactivated by a variety of active ingredients found in EPA-registered chemical germicides that provide low- or intermediate-level disinfection (i.e., ethyl alcohol, isopropyl alcohol, benzalkonium chloride, sodium hypochlorite, ortho-phenylphenol, iodophor) during general use. It is expected that manufacturer-recommended use-concentrations of EPA-registered germicides will be adequate for routine disinfection of cleaned environmental surfaces for management of smallpox care areas. All sterilization methods currently cleared by the FDA for medical instruments and devices will also inactivate these viruses.

For more specific information, refer to the Decontamination Guidelines posted on the CDC web site at: <http://emergency.cdc.gov/agent/smallpox/infection-control/>

## **VIII. Medical Waste**

Medical waste should be contained, subjected to a decontamination treatment, and discarded in accordance with medical waste regulations of the state or other appropriate jurisdiction. This includes the use of offsite medical waste treatment services. However, if healthcare facilities have the capability of treating/decontaminating medical waste onsite, this capacity should be the first option for medical waste management. All currently approved methods of medical waste decontamination can be expected to inactivate pox viruses.