INSTRUCTIONS FOR SPECIMEN COLLECTION FOR ORTHOPOXVIRUS TESTING - (Monkeypox- suspect)

MOLECULAR DIAGNOSTICS LABORATORY
MA STATE PUBLIC HEALTH LABORATORY
305 SOUTH STREET, JAMAICA PLAIN, MA 02130

Stepwise Specimen Collection Instructions: Updated July 26, 2022

Note: Personnel should use contact and droplet precautions (gloves, eye protection, surgical mask (N95 optional unless aerosol generating procedures are being performed), and a gown or disposable covering). Please refer to CDC's website for further guidance on current infection control guidance: https://www.cdc.gov/poxvirus/monkeypox/clinicians/infection-control-healthcare.html. Duplicate swabs are not required to be collected for submission to the CDC.

Materials needed:

- Sterile screw-capped plastic 15 mL tube or small O-ring (1.5-2ml) (black screw top tubes)
- Sterile, dry synthetic swab (including but not limited to polyester, nylon, rayon, or Dacron) with plastic shaft. Do not use cotton tipped, foam swabs, or wooden shaft swabs. **Use of swabs with flexible shafts (e.g. nasopharyngeal (NP) swabs) are not recommended and may yield Inconclusive results due to ineffective swabbing and require recollection.**
- Sterile scalpel or sterile 26-gauge needle (for scab removal only).

Prepare to collect the following diagnostic specimen types (lesions/pustules/scabs in order of preference) from up to two lesions:

- Dry swab of crust and/or fluid from an active, open lesion; or
- Dry swab of an intact vesicle or pustule; or
- Scab.

NOTE: Duplicate samples are no longer required and should NOT be submitted; however, swabs from up to two different lesions per patient may be submitted.

Stepwise Collection Instructions:

1. Don personal protective equipment as described above. **There should be one specimen per tube/collection container only** and ensure the specimen label has the:
   - patient name
   - DOB
   - date of collection
   - site/source of the specimen (e.g., right finger/swab-vesicle fluid)

2. Include the following information on the specimen submission form:
   - Record the same specimen details on the submission form (e.g., right finger/swab-vesicle fluid) as the specimen tube label.
   - Record the symptom onset date as the first day of fever, headache, muscle aches and backache, swollen lymph nodes, chills, exhaustion, or rash. The rash can look like pimples or blisters that appears on the face, inside the mouth, and on other parts of the body, like the hands, feet, chest, genitals, or anus.

3. Procedure to collect different specimen types:
   A. Swab from an open vesicle/pustule with crusts/fluid or an open, wet lesion (preferred):
      1. Use an approved swab type to gently scrape crust material from around a vesicle edge or over a weeping lesion. For a dry, crusty lesion the swab may be moistened with sterile saline. Do not moisten the swab for an open, wet lesion.

2. Place the swab into a dry, sterile 15-mL conical plastic tube, break off the swab handle into a dry, sterile 15-mL conical plastic tube, before securing the lid.
3. Do not add transport medium or any liquid (saline) to the tube.

B. Swab of intact vesicle or pustule:

1. Vigorously swab one closed, fluid-filled lesion (vesicle or pustule) with an approved dry swab and then break off the swab handle into a dry, sterile 15-mL conical plastic tube, before securing the lid. If the vesicle or pustule ruptures, absorb the fluid. Fluid is not required.
2. Do not add transport medium or any liquid (saline) to the tube.

C. Scab:

1. Use a sterile scalpel (or sterile 26-gauge needle) to gently remove the scab and place into a dry, sterile tube, before securing the lid.
2. Do not add transport medium or any liquid (saline) to the tube.

After specimen collection is complete, all protective materials worn by the specimen collector (gloves, mask, gown, etc.) and all used sample collection materials (vacutainer holders, swabs, etc.) should be discarded according to local policies. Needles and scalpels should be disposed of in an appropriate biohazard infectious waste sharps disposal container.

Packaging and Shipping

1. **Complete all fields on the submission form and ensure that the information on the form matches exactly the information on the specimen container. Place the form in the outer pocket of each specimen bag. Each specimen should have its own specimen bag.**
    a. **https://www.mass.gov/doc/speccimen-submission-form/download**
    b. **Unlabeled or mislabeled (information on specimen does not match exactly the information on the form) specimens will be rejected and require recollection.**
2. Samples should be packaged as a Category B. Laboratory testing has indicated that the current monkeypox outbreak is associated with the West African clade of monkeypox virus. The West African clade of monkeypox virus does not meet the definition of Category A infectious substance under the Hazardous Materials Regulations (HMR). Therefore, specimens and material suspected or confirmed to contain the West African clade of monkeypox virus can be shipped as UN 3373 Biological Substance, Category B. Refer to USDOT website for details: https://www.phmsa.dot.gov/sites/phmsa.dot.gov/files/2022-06/Transporting-Infectious-Substances-Safely.pdf
3. Maintain and ship at 4^{O} C. Ship with cold packs. Specimen(s) submitted with wet ice will be rejected. Transport as soon as possible to the Massachusetts State Public Health Laboratory (MASPHL) at 305 South Street, Jamaica Plain 02130. If assistance with immediate transportation is needed, contact 617-983-6800.

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Test Results

- Confirmatory testing of dry swabs of vesicular/pustular fluid; crusts and lesions, and vesicles or scabs will be performed at the MA SPHL with the CDC LRN non-variola orthopoxvirus Polymerase Chain Reaction (PCR) assay. (see result table below)
- If any specimen is positive with the non-variola orthopoxvirus PCR, clade identification will be confirmed by either whole genome sequencing (surveillance only) or clade-specific PCR (surveillance only).
- Inconclusive results may occur if insufficient clinical sample is collected as noted by the inability to detect the human specimen control target.

Result Table

Test Result	Test Interpretation
Positive for Non-variola *Orthopoxvirus*.	Non-variola *Orthopoxvirus* DNA detected by real-time PCR. The assay detects the DNA of common non-variola *Orthopoxvirus* human pathogens, including *Vaccinia*, *Cowpox* and *Monkeypox* viruses. This assay result must be used in conjunction with other diagnostic test results, clinical observations, and exposure history.
Negative for Non-variola *Orthopoxvirus*.	Non-variola *Orthopoxvirus* DNA not detected by real-time PCR primer and probe set.
Inconclusive for non-variola *Orthopoxvirus* DNA by real-time PCR.	An inconclusive result may occur in the case of an inadequate specimen. If patient diagnosis has not been determined, submit additional specimens for analysis.
Equivocal for non-variola *Orthopoxvirus*.	Real-time PCR testing for non-variola *Orthopoxvirus* DNA result is equivocal. An equivocal result may occur in the case of an inadequate specimen or due to cross-contamination during specimen testing. If patient diagnosis has not been determined, submit additional specimens for analysis.

Reasons Specimens Will Be Rejected

Specimen collected with viral transport media or saline. Dry swabs are required.
Specimen received warm (no ice pack).
Specimen received unlabeled. A confirmed link between the specimen and a submission form is not possible. Resubmission requested.
Information on specimen does not match information on submission form.
Specimen container/tube damaged or leaking prior to receipt at laboratory. Resubmission requested.
Missing second identifier on specimen.
Improper specimen type collected. See “Materials Needed” section above for appropriate swab types.