

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

Note: Personnel should wear appropriate barrier protection (N95 or equivalent respirator, gloves, gown, safety glasses/face shield, etc.) for specimen collection of suspected cases of monkeypox.

Specimen Types:

- Dry swab of vesicular/pustular fluid from an intact vesicle or pustule (diagnostic)
- Dry swab of crust and/or fluid from an active, open lesion (diagnostic)
- Roof of vesicle/pustule (diagnostic)
- Scab (diagnostic)
- Throat swab (public health surveillance only)
- Serum (processed- see below) for serology (diagnostic)

Stepwise Collection Instructions:

Donn personal protective equipment described above. Place each specimen labeled with the information below in its own biohazard bag:

- patient name
- DOB
- date of collection
- the site/source of the specimen

Be sure to include the collection site and specimen description on each specimen submission form (e.g., right finger/swab-vesicle fluid). Swabs should be oropharyngeal (throat) size, not nasopharyngeal – smaller swabs may yield insufficient specimens.

To prevent re-collection of swab samples for confirmatory testing by CDC, **collect one other duplicate at a single anatomical site** for any of the three specimen types below (vesicular/pustular fluid swab; open lesion swab; vesicle/pustule roof; or scab) to pair with the serum.

Swab of Vesicular/pustular fluid from intact vesicle or pustule/ De-roofed vesicle or pustule tissue:

Materials needed:

- Disposable scalpels with number 10 blade, or Sterile 26-gauge needle
- Sterile screw-capped plastic 15 mL tube or small O-ring (1.5-2ml) (black screw top tubes)
- Sterile, dry polyester (Dacron) or rayon swab(s) with plastic shaft
- Multiple alcohol wipes

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Procedure:

1. Sanitize the closed fluid-filled vesicle or pustule with an alcohol wipe – allow to dry.
2. Use the disposable scalpel, or the 26-gauge needle, to open the top of the vesicle or pustule.
3. Dispose of the scalpel or needle in appropriate container.
4. Retain vesicle or pustule roof and place in its own tube as a separate sample type.
5. Swab the open area with a dry swab to absorb all the fluid and then break off the swab handle into a dry, sterile 15-mL conical plastic tube, before securing the lid.
6. Take a second sterile polyester or rayon swab and firmly swab the open pustule/vesicle again to obtain a duplicate sample-this sample will potentially be used for confirmatory testing at the CDC.
7. Do not add transport medium or any liquid (saline) to the tube.

Swabs from an open vesicle/pustule with crusts/fluid or an open, wet lesion:

Procedure:

1. Use a dry polyester (Dacron) or rayon swab with a plastic shaft 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.

Scab:

Procedure:

1. Use a sterile scalpel (or sterile 26-gauge needle) to 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.

Throat (oropharyngeal) swab:

Materials needed:

- Sterile, 15 mL screw-capped plastic tube or similar sterile container
- Sterile, dry polyester (Dacron) or rayon swab with plastic shaft

Procedure:

1. Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.
2. Break off end of applicator into a one (1) sterile, plastic tube or similar container and secure the lid.
3. Do not add transport medium or any liquid (saline) to the tube.

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Acute/Convalescent Serum:

Materials needed:

- One (1) Vacutainer holder
- Two (2) Vacutainer needles
- One (1) 10cc red/gray, gold or red-topped serum separator tube for serum collection

Procedure:

1. Collect 5cc of patient blood into plastic red/gray, gold or red-topped serum separator tube.
2. Allow to clot at room temperature for 30 min; centrifuge at 3000 rpm for 15 min.
3. Aliquot 0.5 mL of serum into each of two 1.5 to 2- ml sterile screw-capped plastic tubes with O-ring.

After specimen collection is completed, all protective materials worn by the specimen collector (gloves, mask, gown, etc.) and all used sample collection materials (vacutainer holders, swabs, etc.) must be placed in biohazard bags and autoclaved or incinerated prior to disposal. Needles and scalpels should be disposed of in an appropriate sharps 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.
 - a. <https://www.mass.gov/doc/specimen-submission-form/download>
2. Samples should be packaged as a Category B.
3. Maintain and ship at 4° 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.

Test Results

- Dry swabs of vesicular/pustular fluid; crusts and lesions, and vesicles or scabs will be tested at the MA SPHL by orthopox virus Polymerase Chain Reaction (PCR) assays.
- Throat swab results are for public health surveillance purposes and results will not be reported.
- If any specimen is positive, the duplicate specimen and serum will be sent to CDC for monkeypox virus confirmation and clade identification.