

Practice Point

Monkeypox- Specimen collection and transport guidelines

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The monkeypox virus is an enveloped double-stranded DNA virus that belongs to the Orthopoxvirus genus¹. The main reservoir of poxviruses is the rabbits and non-human primates. Monkeypox causes a smallpox-like illness in humans and can occasionally be transmitted to humans, enabling the incidence of human-to-human transmission².

Although endemic in regions of Africa, there have been a few outbreaks in the United States of America in 2003 and a new world outbreak in 2022.

Most patients' symptoms begin with prodromal sickness with fever, malaise, and enlarged lymph nodes before developing a rash. Individuals may also experience secondary skin and/or soft tissue infection, pneumonitis, ocular problems, and encephalitis.

On 23rd July 2022, the World Health Organization declared the international monkeypox outbreak a global emergency, the highest level of alert the WHO can issue³. This declaration raises public concern about the new pandemic, and because the monkeypox outbreak coincides with the COVID-19 pandemic, there is concern about the severity and mortality of monkeypox cases.

To prevent this outbreak from spreading, testing needs to be augmented, and stringent isolation measures must be put in use.

This comprehensive document emphasizes the practice points involving sample collection, transport, and processing for the monkeypox virus.⁴

1. Procedure for sample collection and transport of the clinical specimen:

Handling the clinical specimens - Personal Protective

Equipment: PPE to be donned before collecting the specimens should include- Coveralls/Gowns, N-95 mask, Face shield/safety goggles, and double pair of gloves. Donning & doffing of PPE should be carefully performed as per the standard procedure.

1.1 Lesion roof, base scrapping, fluid and crust/scab collection [collect from multiple sites]:

- ♣ Before taking the swab, the procedure should be explained to the patient
- ♣ The skin should be sanitized with 80% alcohol wipes to start the collection
- ♣ The lesion roof should be removed using a sterile scalpel or plastic scraper
- ♣ Place the roof in the screw cap plain tube.
- ♣ Similarly, use a 1ml intradermal syringe to collect the pustule/ vesicular fluids from multiple lesions and collect in a new screw cap plain tube.
- ♣ Use the polystyrene swab to collect the scrapings from the base of the lesions by gentle scrapping and put it in a fresh screw cap plain tube
- ♣ The scab/crust, if formed, should also be collected in a new screw cap plain tube using a polystyrene swab.

1.2 Venous Blood Collection in SSGT and EDTA tube:

- ♣ Before taking the swab, the procedure should be explained to the patient.
- ♣ Check the patient's forearm/median cubital fossa for a

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prominent vein. Use the median cubital vein wherever feasible.

- ♣ Apply the tourniquet 4-5 fingerbreadths above the site of venipuncture.
 - ♣ Perform hand hygiene by using an alcohol-based hand rub.
 - ♣ The skin should be disinfected using a wipe containing 70% alcohol, in a circular motion, from the centre to the periphery.
 - ♣ Anchor the vein by holding the thumb below the puncture site.
 - ♣ Insert the needle (vacutainer or syringe needle) into the vein at an angle of about 30°C.
 - ♣ Collect 5 mL of blood (into the syringe or tubes) and aliquot 2 ml in the EDTA tube, and 3 ml in the SSGT tube, for serum separation.
 - ♣ Withdrawing the needle gently, apply a piece of sterile gauze to the puncture site
 - ♣ Invert EDTA tubes gently 4-5 times to ensure proper mixing of the additives.
 - ♣ Tubes should be kept in an upright position on the stand.
 - ♣ Discard the syringe and needle into the appropriate container.
 - ♣ Decontaminate the surfaces of the SSGT and the EDTA tube using 2% Lysol or 0.5-1% sodium hypochlorite wipes
- 1.3 Nasopharyngeal and Oropharyngeal swabs in screw-capped plain tube:
- ♣ Before taking the swab, the procedure should be explained to the patient.
 - ♣ The sterile swab should be removed carefully without contaminating it.
 - ♣ Ask the patient to open their mouth and stick their tongue out.
 - ♣ Using the tongue spatula, the tongue should be pressed down to the floor of the mouth.
 - ♣ Use a sterile polystyrene swab to swab both the tonsillar arches and the posterior nasopharynx without touching the sides of the mouth.
 - ♣ Reaching behind the uvula, the swab should reach the following:
 - a. Tonsillar fauces,

b. The posterior pharynx, and

c. Any ulceration, exudate, lesion, or area of inflammation.

- ♣ Don't let the sterile swab touch the patient's tongue, gums, or teeth as you gently remove it from their mouth.
 - ♣ Place the swab into the screw-capped plain plastic tube [without any medium or VTM]
 - ♣ Similarly, tilt the patient's head back 70 degrees. Gently and slowly insert a polystyrene swab with a flexible shaft through the nostril parallel to the palate until resistance is encountered.
 - ♣ The distance is equivalent to that from the nostril to the ear of the patient, indicating contact with the nasopharynx.
 - ♣ Gently rub and roll the swab, leaving it in place for several seconds to absorb secretions. If a deviated septum or blockage creates difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril
 - ♣ Slowly remove the swab while rotating it. Specimens can be collected from both nostrils
 - ♣ Place the swab into the same screw-capped plain plastic tube [without any medium or VTM] in which the OPS swab was kept
 - ♣ Breaking the extra part of the swab stick, the tube should be recapped tightly.
 - ♣ Keep the tube in an upright position in the stand.
 - ♣ Surface decontaminate the tube using 2% Lysol or 0.5-1% Sodium hypochlorite wipes
- 1.4 Urine sample collection in the screw-capped sterile urine container:
- ♣ Before taking the swab, the procedure should be explained to the patient.
 - ♣ Providing privacy to the patient, the patient should be asked to wipe/clean the genitals.
 - ♣ Ask the patient to collect about 3 to 5 mL of mid-stream urine sample into the collection tube provided, taking care not to contaminate the outside container.
 - ♣ Close the lid carefully and keep the container standing position.
 - ♣ Surface decontaminate the container using 2% Lysol or 0.5-1% Sodium hypochlorite wipes
2. Instructions on sample packaging and transport

Procedures for the transport of the clinical specimens:

- ♣ The samples should be kept at a temperature of 4°C as soon as they are collected.
- ♣ Wearing a full set of appropriate Personal Protective Equipment, identify the labelled sample tubes and seal them with parafilm.
- ♣ The serum tubes should be centrifuged before shipment.
- ♣ All the tubes need to be wrapped with absorbent tissue paper/cotton and placed in Zip lock bags/Secondary receptacles.

Arranging the primary vials within a sturdy, leak-proof secondary container

Option 1: Using a cryo-box as a secondary container. (Seal the box's lid after arranging the samples with cellotape.)

Option 2: Use a 50-ml centrifuge tube as a secondary container. (Seal the neck of the tube using cellotapeNext, placing the zip-lock pouch inside a sturdy plastic container. (Seal the neck of the container using cellotape.)

[Note: Sample vials can also be placed inside a zip-lock pouch, covered in absorbent material and secured by heat-sealing or rubber bands. Then, the zip-lock pouch should be placed inside another plastic pouch and secured.]

Arranging the outer container

Option 1: Use a thermocouple box as an outer container and place the secondary container within it, surrounded by hard-frozen gel packs

Option 2: Use a hard-board box as an outer container and place the secondary container and the gel packs. Placing the completed case inside a leak-proof, zip-lock pouch.

Documents to accompany:

- 1) Packing list/Proforma Invoice
- 2) Air waybill (for air transport) (to be prepared by sender or shipper)
- 3) Value equivalence document (for road/rail/sea transport)

- Securing the zip-lock pouch with the Case Report Form on the outer container

Attaching the labels:

Sender's Address and contact number;

Consignee's Address and contact number;

Emergency Contact's name and number

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