

**Laboratory Preparedness and Response Branch
Biological Response Section**

**Specimen Requirements for Real-Time Polymerase Chain Reaction (PCR) Assay for
the Detection of *Orthopoxvirus* DNA/Non-Variola *Orthopoxvirus* DNA in Clinical
Samples**

Methodology: Real-Time Polymerase Chain Reaction (PCR)

The real-time PCR assay for the detection of *Orthopoxvirus* DNA is for the qualitative presumptive detection of *Orthopoxvirus* DNA in clinical specimens.

The Non-variola *Orthopoxvirus* assay is intended for the in vitro qualitative presumptive detection of non-variola *Orthopoxvirus* DNA in clinical specimens.

Criteria for testing: Only specimens meeting the case definition set by the Centers for Disease Control and Prevention (CDC) and the Disease Outbreak Control Division (DOCD) of the Department of Health will be tested.

Please call:

Oahu (Disease Reporting Line)(808) 586-4586
Maui District Health Office(808) 984-8213
Kauai District Health Office.....(808) 241-3563
Big Island District Health Office (Hilo).....(808) 933-0912
Big Island District Health Office (Kona).....(808) 322-4877
After hours on Oahu(808) 600-3625
After hours on neighbor islands(800) 360-2575
(toll free)

Please refer to the **Medical Advisory** issued on June 3, 2022 ([Attachment](#)).

Testing will commence after approval is received from the DOCD and receipt of appropriate specimen(s).

Acceptable Specimens: Swab of the lesion:
Preferably, swabs should be collected from lesion sites on different locations on the body or from lesions which differ in appearance.

For each lesion site, two matching swabs should be collected. One swab will be processed at the SLD and the

paired swab may be sent to the CDC for further characterization.

Use a sterile swab (see below for types of acceptable swabs) to vigorously swab the base of the lesion – applying enough pressure to collect epithelial cells and to collect vesicular fluid. Swabs must be placed individually into separate, empty tubes to avoid contamination. Place swabs directly into tubes. **Do not add transport medium or any liquid into the tube; the specimen MUST be kept dry.** Tubes must be individually labeled and must be resistant to breakage. While the use gasketed tubes is ideal, the use of any sterile plastic container is acceptable provided specimen containers are properly closed and preferably parafilmmed.

Refrigerate (2-8°C) or freeze (-20°C or lower) samples with-in one (1) hour of collection.

Note: At this time, SLD can NOT accept specimens in VTM or other transport media

For other specimen types not listed, please contact the DOCD or the State Laboratories Division (SLD), Laboratory Preparedness and Response Branch for guidance.

Specimen storage,
and transport:

Use only swabs made from synthetic fibers (including, but not limited to polyester, nylon, or Dacron) with a plastic, wooden or aluminum shaft. Calcium alginate swabs with metal shaft or cotton swabs with wooden sticks are unacceptable because they may cause PCR inhibition.

Samples must be maintained at the temperature they were stored from collection. Refrigerated samples must be maintained at 2-8 °C and shipped on cold packs. Frozen samples must be shipped on dry ice.

Refrigerated samples must be received by the SLD with-in 7 days from collection. If transport will exceed 7 days from collection, it is best freeze samples at -20°C or lower and transport on dry ice.

Follow packing and shipping instructions of the U.S. Department of Transportation (U.S. DOT), and the

International Air Transport Association (IATA) for Biological Substance, Category B.

For further instructions on specimen collection and handling, refer to:
<https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html>
https://www.cdc.gov/csels/dls/locs/2022/06-23-2022-lab-advisory-CDC_Updates_Specimen_Collection_Guidelines_Monkeypox_Virus.html

Criteria for rejection:

- Specimen was not properly collected, or special handling instructions were not followed.
- Use of improper swab(s).
- Unlabeled or incomplete specimen labeling and documentation; Submitter will be notified to provide correct information and corrected SLD Form 81.3.
- Specimen label does not match the requisition.
- Refrigerated samples were received more than 7 days from date of collection.
- Specimens received warm, with no cold ice packs or if frozen, dry ice has dissipated.

Requisition Form:

Each specimen submitted must have a completed SLD Form 81.3. Submitter is responsible for completing SLD Form 81.3 including but not limited to the following information: at least two patient unique identifiers, date of onset of illness, signs and symptoms, travel history, immunization history, test (s) requested, name and address of submitter.

Requisition form(s) shall be placed in a separate bag or the outside pocket of the specimen bag. It shall not be placed with the specimen(s).

Normal Value:

No *Orthopoxvirus* DNA Detected by real-time PCR
Non-variola *Orthopoxvirus* DNA not Detected by real-time PCR

Result Notification:

Laboratory reports will be posted to the DOCD SharePoint. DOCD Investigators will provide reports to submitters.

Test performed at: Biological Response Section (BRS)
Laboratory Preparedness and Response Branch (LPRB)
State Laboratories Division

Contact(s): Remedios Gose at (808) 453-5993 or (808) 554-9992 or
Cheryl Daquip at (808) 453-5984 or (808) 453-6641

Approved By:



AUG 15 2022

Edward Desmond, Ph.D., D(ABMM)
Administrator, State Laboratories Division

Date