

Non-Variola *Orthopoxvirus* – Qualitative, Real-time PCR

Use

This test is intended for detection of non-variola *Orthopoxvirus* in patient samples and as an aid for infection control of non-variola *Orthopoxvirus*.

*Non-variola Orthopoxvirus is a “reportable pathogen” in the State of Oklahoma that **requires immediate reporting of potential cases to the OSDH Infectious Disease Services** via the secure, web-based Public Health Investigation and Disease Detection of Oklahoma (PHIDDO) system or telephone (405-426-8710). Cases must be reported immediately upon suspicion, diagnosis, or positive laboratory test. Information on what and how to report can be found at the Infectious Disease Services [website](#).*

*OSDH Infectious Disease Services **pre-approval is required prior to submission of specimens** from patients with suspected non-variola *Orthopoxvirus* infection to verify the patient meets clinical case criteria. Call 24/7/365 for telephone consultation at 405-426-8710. Once approval for submission has been obtained, please contact the OSDH PHL at 405-564-7750 before shipping.*

Clinical Significance

Monitoring for non-variola Orthopoxviruses is essential for early detection of emerging or re-emerging infections, particularly given recent outbreaks of Mpox (monkeypox) and the potential for zoonotic transmission. Ongoing surveillance allows for rapid identification of cases, timely implementation of infection control measures, and assessment of viral evolution or geographic spread. Continued monitoring also supports public health preparedness by identifying unusual clusters, travel-associated cases, or changes in clinical presentation that may signal shifts in risk. The prevalence of non-variola *Orthopoxvirus* is being monitored by state and national public health services to update preventative measures and limit infections.

Further background information, fact sheets, statistics, and educational resources may be found at the Center for Disease Control [website](#) and at the OSDH Infectious Disease Services [website](#).

Methodology

The Non-Variola Orthopoxvirus Qualitative, Real-time PCR assay is part of the Laboratory Response Network (LRN). It is designed to detect generic non-variola Orthopoxvirus DNA, including Vaccinia, Cowpox, Monkeypox, and Ectromelia viruses, at varying concentrations. The assay does not differentiate between Vaccinia virus or Monkeypox virus and does not detect Variola virus.

Specimen Type

Dry synthetic swabs (including, but not limited to polyester, Nylon, or Dacron) of lesions. Swabs should be separated individually into sterile containers (15 mL conical tubes). **DO NOT add or store swabs in viral or universal transport media.**

Minimum Volume/Size

- **2 swabs per lesion**

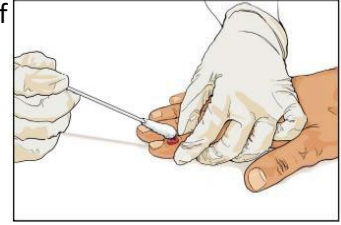
Preferably, select lesions from two different anatomical sites (e.g., 2 swabs from a lesion on the torso and 2 swabs from a lesion on an external appendage).

Collection Instructions

1. Use appropriate personal protective equipment (PPE) in accordance with recommendations for healthcare settings.
2. Label sterile plastic containers with the anatomical sites where specimens will be collected.
3. Collect two swabs from each lesion (in general, 2-3 lesions should be sufficient), preferably from different locations on the body or from lesions that differ in appearance.

- a. Using a sterile, dry synthetic-tipped swab, vigorously swab the surface of the lesion.

Note: Do not use cotton or calcium alginate swabs. Any type of shaft is acceptable (plastic or thin aluminum) as long as it can be broken or cut. Unroofing or aspiration of lesions (or otherwise using sharp instruments for mpox testing) is not necessary, nor recommended, due to the risk for sharps injury.



- b. Put the swab into a dry sterile plastic container. Do not include transport media.
 - c. Using another swab, vigorously swab the surface of the same lesion and place the swab in a separate dry sterile plastic container.
4. Repeat this process on another lesion at a different anatomical site or with different appearance; collect 2 swabs from the lesion and place in separate containers.
 5. Label each primary container with:
 - Patient's first and last name
 - Date of birth
 - Lesion collection site (i.e., face, neck, left hand, etc.)
 - Date of collection
 - Initials of collector
 6. Refrigerate or freeze tubes containing swabs within one hour of collection.
 7. OSDH 419 form must be completed for each collection source and submitted with the specimen to the OSDH PHL.

Additional guidance is available at the CDC website: [Guidelines for Collecting and Handling Specimens for Mpox Testing | Mpox | CDC](#).

Common Causes for Rejection

- Specimens submitted without OSDH Infectious Disease Services approval
- Specimens shipped at ambient temperature (without dry ice or cold packs)
- Swab in transport medium
- Incorrect collection device, e.g., cotton or calcium alginate swab
- Incomplete labeling or documentation of specimens

Shipping

As feasible, ship specimens refrigerated (2-8°C, on frozen cold-packs) or frozen (on dry ice) on the same day as collected. If unable to ship specimens on the same day of collection, specimens must be frozen (at least -20°C but -70°C or colder is preferred) and shipped frozen on dry ice. If site is unable to provide dry ice for shipment, please call the OSDH PHL at 405-564-7750 at least 24 hours ahead of scheduled submission to request dry ice transport. Contact the OSDH PHL at 405-564-7750 to arrange transportation of specimens.

Refer to DOT and IATA shipment standards for proper protocols, packaging, and procedures of biological hazards.

Turn-around Time

Within 2 working days from receipt unless referred to the CDC for further characterization, which may delay availability of final results.

Reference Range

Non-variola *Orthopoxvirus* DNA not detected

Reportable Results

Results are reported to the submitter, as well as the OSDH Infectious Disease Prevention and Response Service for epidemiological purposes.

- Non-variola *Orthopoxvirus* DNA Not Detected
- Presumptive Positive: Non-variola *Orthopoxvirus* DNA Detected; secondary sample referred to CDC for additional testing
- Inconclusive for presence of non-variola *Orthopoxvirus* DNA; if patient diagnosis has not been determined, submit additional specimens for analysis
- Invalid – test is not interpretable. A failed assay may be due to sample quality, PCR inhibition, technical processing.

Interpretation

- Failure to detect non-variola *Orthopoxvirus* DNA suggests an absence of non-variola *Orthopoxvirus* (e.g., Vaccinia, Cowpox, Monkeypox, Ectromelia) in the sample; however, negative results do not preclude infection with non-variola *Orthopoxvirus* or other viruses and should not be used as the sole basis for treatment or other patient management decisions. The assay does not detect Variola (smallpox) virus, the novel *Orthopoxvirus* detected in Alaska in 2015 nor some typical non-human pathogenic *Orthopoxvirus* species, including Raccoonpox virus, Skunkpoxvirus, and Volepox virus. If suspicion remains high, consider submission of additional specimens.
- Detection of non-variola *Orthopoxvirus* DNA suggests the presence of non-variola *Orthopoxvirus* (e.g., Vaccinia, Cowpox, Monkeypox, Camelpox, Ectromelia, Gerbilpox viruses) in the sample; however, the assay does NOT differentiate among these viruses and does not detect. A positive result with this assay in the United States is most likely due to Vaccinia virus but potential exposure to other Orthopoxviruses should be considered. Moreover, a positive result does not imply infectivity or rule out other viral or bacterial co-infections and should not be used as the sole basis for treatment or other patient management decisions. Presumptive results require confirmatory testing.
- An inconclusive test result cannot determine the presence or absence of non-variola *Orthopoxvirus* DNA in the sample. This may be due to presence of a PCR inhibitor, partial target failure or poor-quality specimen. If patient diagnosis has not been determined, submit additional specimens for analysis.

Refer to the Centers for Disease Control and Prevention [MPOX Fact Sheet for Healthcare Providers](#) for interpretation of test results and additional information.

Limitations/Interferences

- Results should always be used in conjunction with epidemiologic and clinical information, plus other laboratory findings.
- As with any molecular test, mutations within targeted sequences could affect primer and/or probe binding resulting in failure to detect the presence of target viruses or newly emerging variants.
- Specimens with insufficient human DNA (cellularity) will produce an inconclusive or invalid result.

CPT Code

87593

Notes

- This test is approved for *in vitro* diagnostic use by the U.S. Food and Drug Administration.