

Monkeypox: Diagnostics – Laboratory Testing

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Disclosures

- Jonathan Gubbay is a consultant scientific editor for Global Infectious Diseases Epidemiology Online (GIDEON).

Laboratory Testing

1. Specimen Collection
2. Specimen packaging/transportation
3. Overview of assays in use at PHO Laboratory
 - Orthopoxvirus real-time PCR
 - Monkeypox virus real-time PCR
4. A review of laboratory testing data to date:
 - Preferred specimen collection guidance.
 - NP/throat swabs and blood are not required in patients with skin lesions
 - A maximum of 3 skin lesion samples are sufficient for reliable detection of monkeypox
5. High enterovirus positivity in children (most with rash do not need monkeypox testing unless direct epidemiological risk factors)


Poxviruses

- Key Genera
 - species:
- Orthopoxvirus
 - Variola (smallpox)
 - Vaccinia virus
 - Cowpox virus
 - Monkeypox virus
 - Camelpox virus
- Molluscipoxvirus
 - Molluscum contagiousum virus
- Parapoxvirus
 - Orf virus (sheep, goats)
 - Pseudocowpox virus (milker's nodule)



Orf virus infection on the hand of a person with a weak immune system.

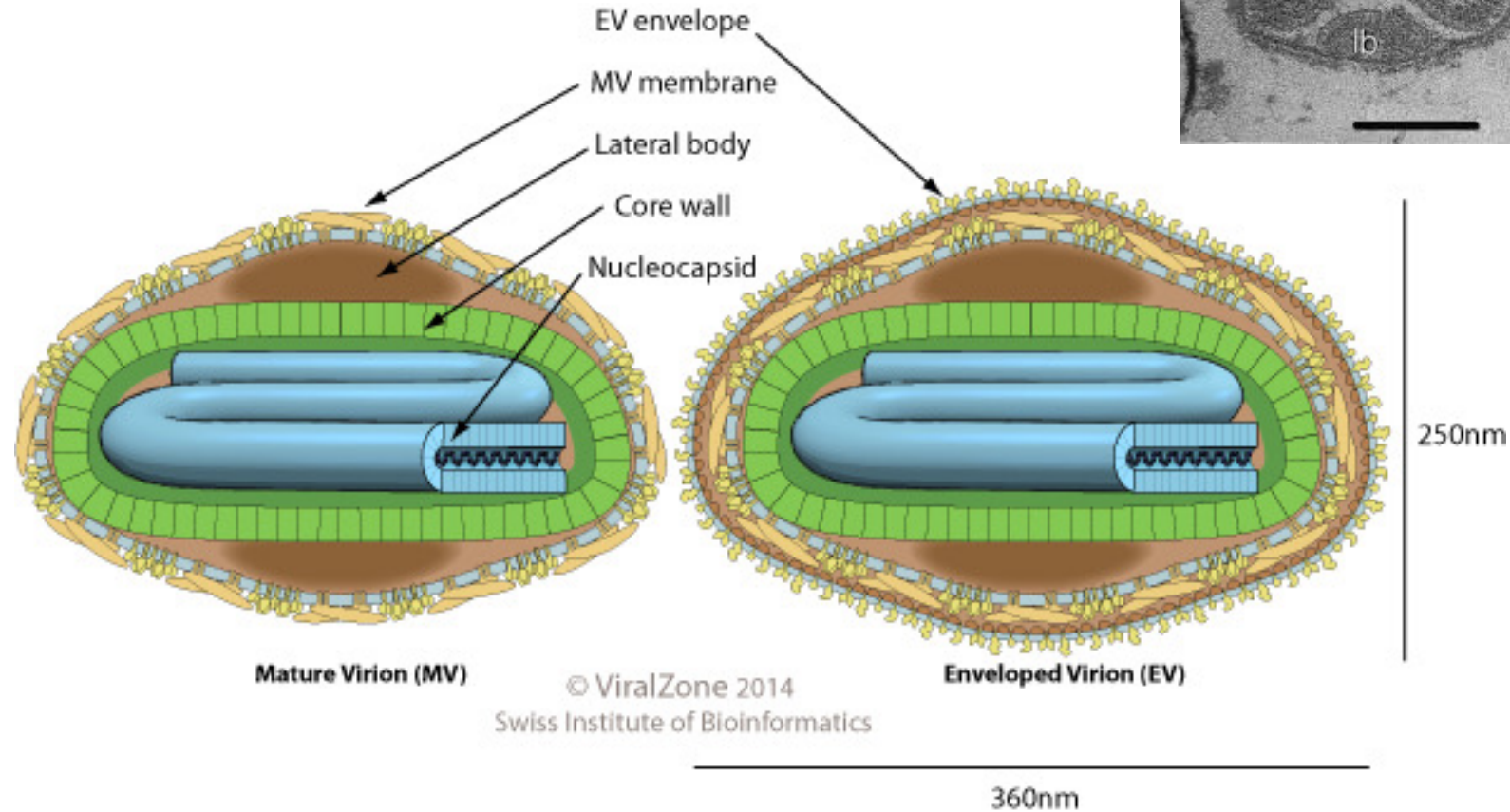
<https://www.cdc.gov/poxvirus/orf-virus/people.html#:~:text=Orf%20virus%20infection%20on%20the,will%20become%20ulcerative%20in%20nature.>



Pseudocowpox
is a viral disease that
causes sores on cow teats
and human hands.

Cows teat photo from "Countdown Downunder" at http://www.countdown.org.au/Teat_images.htm
Hand photo from Swiss Medical Weekly at <http://www.smw.ch/oeiv/1998/128-38-085-98.html>

Orthopoxvirus



- Enveloped, brick-shaped virus about 250 nm long
- Linear, dsDNA genome of 170-250 kB

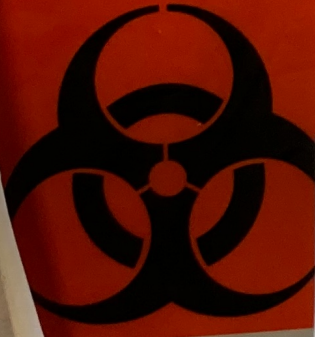
Sample Collection Kit

MONKEYPOX VIRUS
TESTING KIT

OR INTERDEPARTMENTAL TRANS
OF LABORATORY SAMPLES

INSTRUCTIONS

PLACE SPECIMEN TO BE TRANSPORTED IN
RESEALABLE PORTION OF BAG.
INCLUDE SPECIMEN DOCUMENTATION IN
BAG.



TRANSPORT INTERDÉPART
CONTENANTS DE LABOR

INSTRUCTIONS

PLACE SPECIMEN À TRANSPORTER
DANS LA PARTIE RESEALABLE DU SAC.
INCLURE LA DOCUMENTATION DU
SPECIMEN DANS LE SAC.

Public Health Ontario | Santé publique Ontario

For laboratory use only
Date received (yyyy/mm/dd): PHOL No.:

General Test Requisition

ALL Sections of this form must be completed at every visit

1- Submitter	2 - Patient Information
Name Address City & Province Postal Code	Health Card No.: Sex: <input type="radio"/> Male <input type="radio"/> Female Date of Birth (yyyy/mm/dd): Medical Record No.: Last Name per health card: First Name per health card:
Clinician Initial/Surname and OHIP/CPSO No.:	Address:
Telephone: Fax:	Postal Code: Phone Number:
cc Doctor / Qualified Health Care Provider Information	Submitter Lab No.:
Name: Tel:	Public Health Unit Outbreak No.:
Lab / Clinic Name: Fax:	Public Health Investigator Information
PHOL No.:	Name:

Monkeypox Sample Collection Guidelines

The following samples should be collected on patients under investigation for Monkeypox virus

- Nasopharyngeal Swab
- Lesion roof (x2)
- Swab of lesion fluid (x2)

Each test kit contains:

- 3 viral UTM tubes (pink media) + swabs
- 2 red-cap 50 ml falcon tubes
- 1 Public Health Ontario requisition with test details filled in
- 2 alcohol wipes

Obtain a disposable scalpel or sterile 26-gauge needle before entering the room

NOTIFY ON-CALL IPAC & ON-CALL MICROBIOLOGIST BEFORE STARTING COLLECTION

Nasopharyngeal swab

Procedure:

1. Collect nasopharyngeal swab in a similar manner as is done for respiratory virus testing.
2. Place the swab in the UTM tube (pink media) and break off swab end.



Courtesy of Rob Kozak, Sunnybrook Health Sciences

Sample Specimen Collection Guidelines: Sunnybrook Health Sciences

Monkeypox Sample Collection Guidelines – June 28 2022

The following samples should be collected on patients under investigation for Monkeypox virus

- Swab of lesion fluid (x2)
- Lesion roof (x2)

The following samples can be collected on patients under investigation for Monkeypox virus if indicated clinically

- Nasopharyngeal Swab – *Note:* a throat or oral/nasal (back of tongue, cheek and two nares) can be done for patient comfort if indicated as an alternative using the same swab and media
- Serum – should be collected in all febrile patients and would require >0.5 mL in gold top tube

Each test kit contains:

PLACE REQUISITIONS IN POCKET OF LARGE BIOHAZARD BAG AND ALL INDIVIDUALLY BAGGED SAMPLES IN LARGER BIOHAZARD BAG AND SEND TO CORE LAB (CG-01) USING PORTER. DO NOT SEND SAMPLES BY THE TUBE SYSTEM

Example Specimen Collection Guidelines: Sunnybrook Health Sciences

NOTIFY ON-CALL INFECTIOUS DISEASES STAFF BEFORE STARTING COLLECTION

Lesion Roof and Fluid Swab Collection

1. Sanitize lesion with an alcohol wipe, allow to dry.
2. Use a disposable scalpel (or a sterile 26 Gauge needle) to open and remove the roof of the intact vesicle or pustule (do not send the scalpel or needle but dispose of them in appropriate container).
3. Place the lesion roof in sterile red cap 50ml Falcon tube.
4. After removing lesion roof the base should be vigorously swabbed with a swab included in the kit.
5. Place swab in the viral transport media tube (clear media) and break off the swab end.
6. Label tube with patient information sticker with skin location written on sticker
7. Place samples in biohazard bag with requisition indicating sample site location
8. Repeat above steps to collecting from a second lesion

PLACE REQUISITIONS IN POCKET OF LARGE BIOHAZARD BAG AND ALL INDIVIDUALLY BAGGED SAMPLES IN LARGER BIOHAZARD BAG AND SEND TO CORE LAB (CG-01) USING PORTER. DO NOT SEND SAMPLES BY THE TUBE SYSTEM

Guidance is changing: CDC is a bit different to Canada

Preparation and Collection of Specimens

Updated June 29, 2022 [Print](#)

Two swabs from each lesion should be collected for testing. Using two sterile synthetic swabs (including, but not limited to polyester, nylon, or Dacron) with a plastic, wood, or thin aluminum shaft, swab the lesion vigorously to collect adequate DNA. Do not use cotton swabs. It is not necessary to de-roof the lesion before swabbing. Break off the end of each swab's applicator into a 1.5- or 2-mL screw-capped tube with O-ring or place the entire swab in a sterile container that has a gasket seal and is able to be shipped under the required conditions. Two swabs from each lesion should be collected, preferably from different locations on the body or from lesions which differ in appearance. Swabs and other specimens should each be placed in different containers. If using transport media, only VTM is accepted at CDC at this time; do not use universal or other transport media. <https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html>

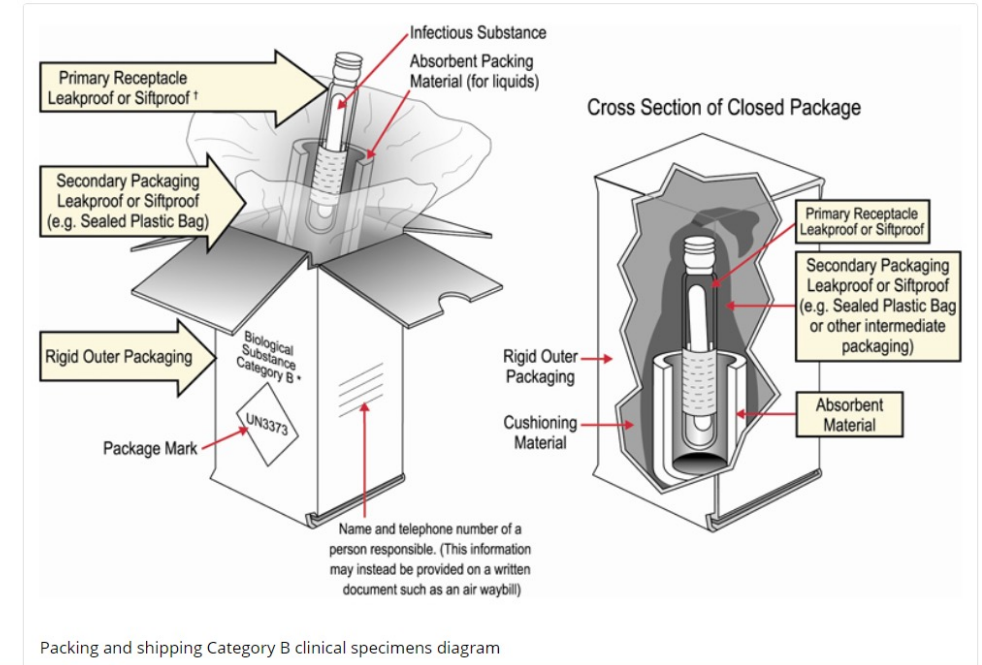
- I would de-roof a lesion before specimen collection
- I would collect one swab per lesions if multiple lesions;
 - Two swabs from the same lesion if only one lesion present.

Specimen packaging

- Specimens can be collected in any clinical setting where appropriate PPE/infection control precautions can be instituted.
- Transport Canada has temporarily reclassified clinical specimens from patients undergoing Monkeypox testing as **UN3373 Biological Substance, Category B for land transport**. This is the same packaging that is currently in place for transporting any specimen from a patient with a suspected infection (e.g. NP swab, vaginal swab, blood cultures).

Category B Infectious Substance

Package, label, and ship low- or moderate-risk specimens as a **Category B** infectious substance (UN 3373) in accordance with the U.S. Department of Transportation's Hazardous Materials Regulations and the International Air Transport Association Dangerous Goods Regulations.



In addition to the routine Category B requirement, the outer packaging must be marked, on a contrasting background, with “TU 0886”, “Temporary Certificate – TU 0886” or “Certificat Temporaire – TU 0886”. For full details on packaging and transporting, see the [Temporary Certificate TU 0886](#).

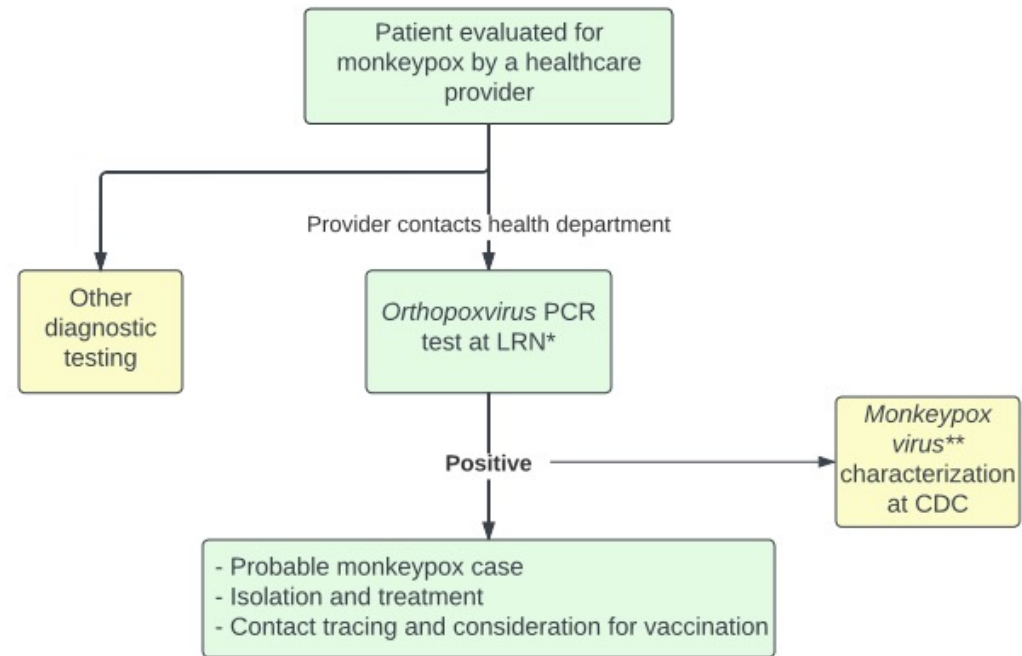
Specimen transportation to the laboratory

- The same courier systems that currently pick up specimens for microbiology testing from healthcare provider practice locations are utilized for transporting monkeypox specimens to the testing laboratory.

Laboratory Tests: *Orthopoxvirus* PCR

- *Monkeypox virus* is in the *Orthopoxvirus* genus of *Poxviridae* family.
- Other Orthopoxviruses include *Smallpox*, *Vaccinia*, *Cowpox*, *Camelpox*.
- Currently *Monkeypox virus* is the only *Orthopoxvirus* detected in Canada.

Diagnostic Process for *Monkeypox Virus* Testing



*PCR - polymerase chain reaction; LRN - laboratory response network

**Monkeypox is a member of the orthopox group of viruses.

Important Links and Contacts | [Information for Laboratory Personnel](#) | [Preparation and Collection of Specimens](#) | [Emergency Operations Center: 770.488.7100 \(Available 24/7\)](#) | LRN contact: Use the 'Contact Us' feature on the LRN website, Or LRN@cdc.gov | [Public Health contact list to pursue LRN lab testing](#)

[Diagnostic Process for Monkeypox Virus Testing \(cdc.gov\)](#)

Orthopoxvirus PCR: PHO Laboratory Sample Report

- Initial assay used at PHOL, up until June 22

Test	Result	Date Approved
Orthopoxvirus PCR	Detected	2022-06-29
Interpretation	Orthopoxvirus DNA detected by real-time PCR.	2022-06-29

Note: *Monkeypox virus is the only virus belonging to the Orthopoxvirus genus recently detected in Canada.* ✓
This test was performed using a Canadian Laboratory Response Network assay verified at Public Health Ontario Laboratory for clinical testing.

Copy of results sent to MOH.

- Initially all specimens were sent to NML for parallel testing.
- Once enough negative specimens were tested by NML, only positives and indeterminates were forwarded for NML retesting.
- As of June 23, Orthopoxvirus PCR results at PHOL are final.

Introduction of a *monkeypox virus* real-time PCR assay June 23, 2022

- Assay developed at US CDC
- Currently it is the only test done on most specimens.
- 2 targets – run as a multiplex (same test well)
 - Monkeypox target
 - West African clade target.

Journal of Virological Methods 169 (2010) 223–227

Contents lists available at ScienceDirect

Journal of Virological Methods

Short communication

Real-time PCR assays for the specific detection of monkeypox virus
West African and Congo Basin strain DNA

Yu Li*, Hui Zhao, Kimberly Wilkins, Christine Hughes, Inger K. Damon

Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology (Proposed), National Center for Emerging and Zoonotic Infectious Diseases (Proposed),
Centers for Disease Control and Prevention, Atlanta, GA, United States

<https://pubmed.ncbi.nlm.nih.gov/20643162/>

Monkeypox specific PCR

- Each of the 2 targets (Monkeypox; West African clade Monkeypox) are reported separately on all specimens.
- Detection of either Monkeypox target is sufficient for laboratory confirmation of Monkeypox virus infection.
- Results can be Detected, Indeterminate, Not Detected, Invalid.

This is a laboratory developed test endorsed by the World Health Organization, which has been verified at Public Health Ontario Laboratory for clinical testing.

This assay includes two targets - a generic Monkeypox virus (panmonkeypox) target that detects both Monkeypox virus clades [West African and Congo Basin (Central African)], and a target which only detects the West African clade.

Detection of either Monkeypox virus PCR target is sufficient for laboratory confirmation of monkeypox virus infection.

Copy of results sent to MOH.

Detected: Cycle threshold (Ct) ≤ 38

Indeterminate: Ct 38.01 to 39.99

Not Detected: Ct ≥ 40

Monkeypox specific PCR

- Each of the 2 targets (Monkeypox; West African clade Monkeypox) will be reported separately on all specimens.
- Detection of either Monkeypox target is sufficient for laboratory confirmation of Monkeypox virus infection.
- Results can be Detected, Indeterminate, Not Detected, Invalid.

Test	Result	Da
Monkeypox virus PCR	Indeterminate	20%
West African Clade Monkeypox virus PCR	Indeterminate	20%
Interpretation	Monkeypox virus (panmonkeypox) DNA target INDETERMINATE; West African clade Monkeypox virus DNA target INDETERMINATE by real-time PCR.	20%

Note: This result may be due to low viral target quantity (cycle threshold 38.01-39.99) in the clinical specimen approaching the limit of detection of the assay, or may represent nonspecific reactivity (false signal) in the specimen. Please submit additional specimen(s) for testing if clinically indicated.

Monkeypox specific PCR

- Each of the 2 targets (Monkeypox; West African clade Monkeypox) will be reported separately on all specimens.
- There is a risk of false-positive results when low pretest probability patients are tested. These are usually high Cycle threshold – positive.

Test	Result	Date
Monkeypox virus PCR	Detected	202
West African Clade Monkeypox virus PCR	Not Detected	202
Interpretation	Monkeypox virus (panmonkeypox) DNA target DETECTED; West African clade Monkeypox virus DNA target NOT DETECTED by real-time PCR.	202

Note: This result may be due to low viral target quantity (cycle threshold ≥ 35) in the clinical specimen approaching the limit of detection of the assay, or may represent nonspecific reactivity (false signal) in the specimen. Please submit additional specimen(s) for testing if clinically indicated.

Monkeypox specific PCR

- PHO Monkeypox PCR results are final – no requirement for routine retesting at NML.
- Orthopox PCR and/or gene fragment sequencing may be used as secondary test/s as required.
- One positive specimen from each patient will be forwarded to Canada's National Microbiology Laboratory for whole genome sequencing (for surveillance, no individual report anticipated)

2. PHO Laboratory Monkeypox Testing Review (May 22 to June 26, 2022)



Table Detection results* for MPXV by qPCR in clinical specimen types submitted to the Public Health Ontario Laboratory

Specimen types and overall No.	Blood (N=190)			Nasal / NPS (n=137)			Throat / OP (n=106)			Skin / Lesions (n=559)			Urine (N=41)		
Number (% positive)	29 (15.3%)			20 (14.6%)			31 (29.2%)			177 (31.7%)			6/41 (14.6%)		
Specimens from positive patients: pos/total (% pos)	29/67 (43.2%)			20/36 (55.6%)			31/46 (67.4%)			177/213 (83.1%)			6/21 (28.6%)		
Target	Opx	Mpx	WA	Opx	Mpx	WA	Opx	Mpx	WA	Opx	Mpx	WA	Opx	Mpx	WA
No. Positive	26	15	15	16	11	13	24	13	13	135	74	75	5	4	4
Ct mean	38.6	35.9	35.3	36.3	32.4	32.8	32.0	27.8	27.3	27.1	23.1	23.1	37.3	32.2	32.2
Ct Standard Deviation	4.1	2.1	2.3	6.3	5.6	5.6	6.5	5.1	4.5	7.3	6.5	6.6	5.1	5.5	5.1

Abbreviations: Opx, Orthopoxvirus test; Mpx, pan monkeypox specific target; WA, West African clade specific target; OP, Oropharyngeal; Pos, positive; Ct, cycle threshold

*Indeterminate results are not include above. They are as follows for various sample types; blood 6/190 (3.2%), Nasal/NPS 5/137 (3.6%), throat/OP 2/106 (1.9%), skin lesion 10/559 (1.8%) and urine 6/41 (14.6%)

- Urine – 6/41 (14.6%) positive – 21/41 were submitted from monkeypox-positive patients.
- Semen – 2/5 (40%) positive – all 5 submitted from monkeypox-positive patients.
- Saliva – 1/4 (25%) detected. One from a monkeypox-positive patient.

Skin is the most sensitive specimen – 83.1% detected, 1.8% indeterminate, among positive patients.

Throat swabs may be better than NP swabs (no data on presence of oral lesions which may explain higher positivity)

Preferred Specimen Guidance – NP or blood is not needed if collecting skin specimens.

- All patients with positive blood specimens had other positive specimens
- Among 78 monkeypox confirmed cases with both NPS/throat (= NPS or throat) and skin specimens submitted for testing:
 - Skin was detected in 72 (92.3%); NPS/throat detected in 44 (56.4%).
 - NPS/throat AND skin were detected in 38/78 (48.7%).
 - Skin detected, NPS/throat not detected in 34/78 (43.6%)
 - NPS/throat detected, skin not detected in 6/78 (7.7%)

Preferred Specimen Guidance – NP or blood is not needed if collecting skin specimens.

NP and blood are not required if skin /lesion samples can be collected (i.e. open or vesicular/pustular skin lesions).

- NP/throat/blood most utility in prodromal phase of illness, or early rash (macular, papular)
- NP/throat can be helpful if considering enterovirus or respiratory viruses, in particular in children.

Preferred Specimen Guidance – Up to 3 skin lesion specimens is adequate.

- Sensitivity of an individual skin specimen approaches 85%.
 - A maximum of of 3 skin specimens is sufficient for very sensitive detection of monkeypox (no need to swab every single skin lesion).

For Monkeypox Testing, Use Lesion Swab Samples to Avoid False Results: FDA Safety Communication



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Email



Print

Date Issued: July 15, 2022

The U.S. Food and Drug Administration (FDA) is advising people to use swab samples taken directly from a lesion (rash or growth) when testing for the monkeypox virus. The FDA is not aware of clinical data supporting the use of other sample types, such as blood or saliva, for monkeypox virus testing. Testing samples not taken from a lesion may lead to false test results.

<https://www.fda.gov/medical-devices/safety-communications/monkeypox-testing-use-lesion-swab-samples-avoid-false-results-fda-safety-communication>

Children are more likely to have enterovirus than any other virus

- PHOL routinely tests all specimens from children (<18yo) for enterovirus
 - adults are tested on request.
- Enterovirus was detected in 25 (71.4%) of 35 children tested.
 - Also detected in 7 (47%) of 15 adults tested.
- Children with rashes should not be routinely tested for monkeypox unless there is an epidemiological link with a case of monkeypox.

Toronto

Ontario doctors warn there's a surge of hand-foot-and-mouth disease in kids and parents



Spike in cases is puzzling because it's happening during the summer, doctors say



Clara Pasiaka · CBC News · Posted: Jul 07, 2022 4:00 AM ET | Last Updated: July 7



<https://www.cbc.ca/news/canada/toronto/hand-foot-mouth-disease-2022-1.6511796#:~:text=Hand%2Dfoot%2Dand%2Dmouth%20disease%20is%20communicable%20illness%2C,Loss%20of%20appetite.>

Monkeypox is a Disease of Public Health Significance

Ministry of Health

Ontario Public Health Standards:
Requirements for Programs, Services and Accountability

Infectious Disease Protocol

Appendix 1: Case Definitions and Disease- Specific Information

**Disease: Smallpox and other
Orthopoxviruses including
Monkeypox**

Effective: June 2022

All persons with suspected monkeypox must be reported to their local public health unit.

https://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/smallpox_chapter.pdf

PHO Monkeypox Resources

Public Health Ontario Monkeypox Test Information Sheet

<https://www.publichealthontario.ca/en/laboratory-services/test-information-index/monkeypox-virus>

PHO Monkeypox Resources:

<https://www.publichealthontario.ca/en/Diseases-and-Conditions/Infectious-Diseases/Vector-Borne-Zoonotic-Diseases/Monkeypox>

This screenshot shows the 'Monkeypox Virus' page on the Public Health Ontario website. At the top, there is a navigation bar with the PHO logo and a search box. Below the navigation bar is a blue alert banner with a white exclamation mark icon and the text: 'Alert MyPHO users are currently unable to update subscription preferences. We are working to resolve the issue.' The main content area features a breadcrumb trail: 'Welcome > Laboratory Services > Test Information Index > Monkeypox Virus'. To the right of the breadcrumb trail are icons for 'Save', 'Share', and 'Print'. The page title is 'Monkeypox Virus'. Below the title is a table with three columns: 'Testing Indications', 'Specimen Collection and Handling', and 'Requisitions and Kit Ordering'. The 'Testing Indications' column contains 'Test Frequency and Turnaround Time (TAT)'. The 'Specimen Collection and Handling' column contains 'Reporting'. The 'Requisitions and Kit Ordering' column contains 'Test Methods'. Below the table is a section titled 'Testing Indications' with a paragraph of text: 'This document provides testing information for Monkeypox virus (Poxviridae family, Orthopoxvirus genus). For testing information of poxviruses other than Monkeypox virus and Variola virus, please refer to the following link:'

This screenshot shows the 'Monkeypox' page on the Public Health Ontario website. At the top, there is a navigation bar with the PHO logo and a search box. Below the navigation bar is a blue alert banner with a white exclamation mark icon and the text: 'Alert MyPHO users are currently unable to update subscription preferences. We are working to resolve the issue.' The main content area features a breadcrumb trail: 'Welcome > Diseases & Conditions > Index > Infectious Diseases > Vector-Borne and Zoonotic Diseases > Monkeypox virus'. To the right of the breadcrumb trail are icons for 'Save', 'Share', and 'Print'. The page title is 'Monkeypox'. Below the title is a paragraph of text: 'Monkeypox is a rare viral illness that causes fever, headache, swollen lymph nodes and lethargy, followed by the development of a rash over a person's body. It is spread to people through direct contact with the bodily fluids or lesions of infected animals or people, via respiratory droplets from an infected person, or from mother to fetus.'

Acknowledgements

- Public Health Ontario Laboratory staff.
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- Ontario Ministry of Health
- Ontario's Network of Microbiology Laboratories.