Mpox (Monkeypox virus infection)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for Mpox.

Version	Status	Authorisation	Consensus Date
1.5	Updates to laboratory diagnosis and specimen handling content. Inclusion of recommendations for testing asymptomatic individuals.	PHLN	7 March 2025
1.4	All references to 'Monkeypox' disease amended to 'Mpox'. Update to laboratory case definition template.	PHLN	17 July 2023
1.3	Updated to include individual laboratory risk assessments to determine appropriate personal protective equipment to use when handling MPX specimen.	PHLN	17 August 2022
1.2	Updates to the probable/confirmed and suspected MPX guidelines.	PHLN	1 July 2022
1.1	Updates to the personal protective equipment recommendations.	PHLN	27 June 2022
1.0	Initial PHLN Laboratory Case Definition	PHLN	27 May 2022

1 PHLN summary laboratory definition

1.1. Condition

Acute mpox due to infection with the monkeypox virus.

1.1.1 Definitive Criteria

- Detection of monkeypox virus by nucleic acid amplification testing in clinical specimens; OR
- Detection of monkeypox virus-specific sequences using next generation sequencing for clinical specimens; OR
- Isolation of monkeypox virus by culture from clinical specimens

1.1.2 Suggestive Criteria

- Detection of Orthopoxvirus by nucleic acid amplification testing in clinical specimens; OR
- Detection of Orthopoxvirus by electron microscopy in clinical specimens

1.1.3 Comments

In addition to testing for monkeypox virus, referring clinicians should consider testing for other infective causes of vesicular, vesiculopapular and pustular rashes. Other differential diagnoses may include varicella zoster virus, herpes simplex virus-1 and -2, syphilis, molluscum contagiosum and Orf virus. Smallpox (caused by the variola virus) has been eliminated as a naturally occurring infectious illness with global eradication certified by the World Health Organization (WHO) in 1980 but bears clinical similarities to monkeypox virus infection. If smallpox is considered a differential diagnosis, there are additional public health considerations, and specimens should be handled as for a suspected smallpox case; please refer to the PHLN Smallpox Laboratory Case Definition.

Variola, vaccinia, cowpox, monkeypox and other Orthopoxvirus virions cannot be definitively distinguished from each other by electron microscopy.

2 Introduction

Monkeypox virus (MPXV) is a poxvirus (Family Poxviridae, genus Orthopoxvirus; other members include variola and vaccinia viruses) with brick-shaped virions of 220–450 nm in length [1]. Two clades of monkeypox virus have been identified, Clade I (formally the Congo Basin (Central African) clade) and Clade II (formally the West African clade) [2]. There are also subclades and lineages which have different epidemiology and clinical features.

Monkeypox virus is considered the most important Orthopoxvirus capable of infecting humans since the eradication of smallpox (caused by variola virus).

Mpox is a zoonosis, MPXV can infect a range of mammalian species (including humans and non-human primates), but the natural reservoir remains unknown, although arboreal rodents including rope squirrels, sun squirrels, African pouch rats and dormice have been suggested as the most probable [3,4]. Most cases have been reported in the Democratic Republic of the Congo (DRC) and other central and western African countries. Mpox cases reported outside Africa have been linked to international human travel or movement of animals, and secondary transmission has been reported [5-7]. In 2022, a large number of mpox cases due to MPXV clade IIb were reported in Europe, the United Kingdom (UK), and the Americas (including the United States of America (USA), Canada, Brazil, Colombia, Mexico and Peru) in men who have sex with men (MSM) [8,9]. There were 144 cases of mpox reported in Australia in 2022 [10]. Since late 2023, a large number of mpox cases due to MPXV clade Ib have occurred in DRC with spread to other countries within and outside Africa (including Sweden, Thailand, India, Germany, UK, USA and Canada). However, there have been more than 1400 mpox cases in Australia in 2024 [10], with almost all cases from local transmission.

Mpox has a usual incubation period of 7–14 days (but can range from 5-21 days). The initial phase of clinical illness (prodromal period) usually lasts 1 to 5 days and is characterised by fever, fatigue, lymphadenopathy, headache, muscle aches, joint pain and back pain [3,11].

This is typically followed by a second phase involving the appearance of a rash lasting 2–3 weeks. The skin lesions typically begin to develop simultaneously and evolve together on any given part of the body, including the oral mucous membranes, conjunctiva, cornea and genitalia, and may be generalised or localised, discrete or confluent. In a minority of cases non-simultaneous cropping can occur, as for varicella. The evolution of lesions progresses through four stages — macular, papular, vesicular, to pustular — before scabbing over and then healing.

In MPXV clade IIb disease, previously atypical locations of infection such anogenital and mucosal lesions were common. Other features such as localised rash without prodromal symptoms and anorectal pain from proctitis without other clinical features have also been observed. Clinical presentation may also be attenuated by prior infection and/or vaccination. Care is largely supportive. The case fatality rate in mpox patients is estimated to be between 0–11% [12]. However, there are significant challenges in accurately estimating this rate [3]. Case fatality rates are lower for patients infected with clade IIb and clade Ib viruses (<1%)

compared to clade Ia (5-10%) viruses. Clinical presentation is similar to smallpox, although the development of lymphadenopathy is generally not seen with smallpox.

Human-to-human transmission is by close physical contact, contaminated fomites and respiratory droplets [12-15]. Transmission can also occur from direct contact with infected animals.

Vaccinia vaccination using a live-virus preparation of vaccinia virus (ACAM2000™) is cross-protective against Orthopoxvirus infection and was used for widespread vaccination against smallpox. Epidemiological evidence suggests that vaccinia vaccination has some protection against mpox [16,17]. In the US, (MVA-BN) JYNNEOS^{Ò}, a newer vaccinia-based, live attenuated vaccine was specifically licensed for the prevention of mpox [18], and this vaccine was also rolled out in Australia in 2022. Cessation of vaccinia vaccination worldwide (including in Australia) following smallpox eradication means that populations remain largely susceptible to MPXV infection.

3 Laboratory diagnosis

3.1 Test method

3.1.1 Nucleic acid amplification tests

Multiple in-house nucleic acid amplification (NAA) assays have been described for detection of Orthopoxviruses including MPXV using both gel-electrophoresis based and real time methods [1]. Commercial assays for the specific detection of MPXV are available overseas and in Australia [19,20]. At the time of writing, one commercially supplied test kit for the detection of MPXV is registered on the Australian Register of Therapeutic Goods (ARTG) (ARTG 407409). In-house and commercial assays typically target the F3L, G2R or TNF receptor genes. Evaluations of some of these assays show comparable limits of detection [21]. Multiplex NAA assays which differentiate Clades I and II of the MPXV have been described [22], based on fragment length of the NAA product of the E5R gene. More recently, NAA assays have been developed to detect clade Ib MPXV with the 1140 base pair deletion in the C3L gene [23]. As the analytical sensitivity of NAA assays used in the detection of Orthopoxviruses is high [24], they may potentially be used to exclude a diagnosis of MPXV infection from an appropriately collected sample. Where Orthopoxvirus DNA is detected, MPXV-specific NAA assays should be performed to confirm the diagnosis.

3.1.2 Viral genome sequencing

Genome sequencing from clinical samples with sufficient viral load can assist in determining MPXV clades, subclades and lineages. In addition, sequencing may help identify or rule out transmission chains, provide contextualisation for resolving new international incursions or local circulation [25], inform whether NAA assays are fit for purpose, and monitor for therapeutic and vaccine escape [26]. Genomic sequencing also allows the monitoring of ongoing viral evolution in the human population [27]. Both Illumina and Oxford Nanopore sequencing technologies have been used for whole genome sequencing [28]. Amplicon based, metagenomics or targeted enrichment by hybdrisation capture sequencing methods have been used. Global genomic data are available on publicly accessible databases such as GISAID EpiPox.

3.1.3 Electron microscopy

Electron microscopy can distinguish orthopoxvirus infection from parapoxviruses and herpesviruses but is unable to differentiate species within the genera. For species level identification, more specific methods such as NAA techniques are required. Orthopoxviruses have a distinct brick-shaped morphology when examined with sodium phosphotungstate or other heavy-metal negative stains.

3.1.4 Virus growth assays

Orthopoxviruses can be isolated in a variety of cell lines including Vero, African Green monkey, Buffalo Green Monkey and HeLa cells. Successful isolation of virus is generally confirmed using NAA in addition to observing cytopathic effects. The analytical performance of virus culture using different cell lines is uncertain, and is performed at reference laboratories only under PC3 conditions by staff vaccinated against vaccinia or smallpox. In laboratories performing MPXV isolation, standardised protocols (including for virus harvesting) should be developed. Further characterisation of the isolate and monitoring of the serially passaged virus by genomic sequencing may be performed.

3.1.5 Serology

Serological methods for diagnosis of Orthopoxviruses have been described including virus neutralisation tests, haemagglutination-inhibition, immunofluorescence, enzyme-linked immunosorbent assays and Western blot. There are currently no assays registered on the ARTG.

Serological testing may have a role in diagnosis of acute mpox infections, epidemiologic surveillance studies and also to determine the secondary attack rate in asymptomatic or pauci-symptomatic contacts of cases [29]. Testing for MPXV-specific IgM can be considered within five days of presentation, or IgG after eight days [30]. An IgM capture assay [31] was found to have sensitivity and specificity of 95% in detecting epidemiologically linked and laboratory confirmed cases.

Orthopoxviruses have a high degree of antigenic relatedness and serologic tests for MPXV may be cross-reactive with other orthopoxviruses, including vaccinia virus-based vaccines [29,31]. Neutralising antibodies to vaccinia virus have been detected at least 20 years post vaccination. Vaccination and infection history should be considered in interpretation of any MPXV serology.

3.2 Suitable specimen types

At present it is not recommended to test for MPXV in asymptomatic individuals at high risk of infection as there are insufficient data on the utility or cost effectiveness of screening for mpox. Similarly pre-travel MPXV testing in asymptomatic persons is also not recommended.

Lesion material should be collected from persons with suspected MPXV infection with an active lesion or rash. Acceptable sample types include lesion fluid, lesion tissue, lesion crust or skin biopsy [3]. It is recommended at least two swabs from morphologically distinct lesions and/or anatomical locations are collected. Material can be collected using a sterile dry swab (e.g. nylon, polyester or Dacron) suitable for NAA testing.

Nasopharyngeal and throat swabs are also suitable specimens. NAA of blood may be considered in specific cases. Whole blood or serum samples can be tested by NAA to detect the presence of MPXV, but are often negative due to the transient nature of viraemia [32], and therefore should not be used to exclude mpox. A minimum of 5 mL of EDTA whole blood or 10 mL of serum is recommended.

If there is no obvious lesion, for example, macular rash alone, discuss approaches with a specialist microbiologist. If symptomatic mucosal disease is suspected, a rectal swab (proctitis), urethral swab or first void urine (urethritis) and conjunctival (conjunctivitis) swab, as appropriate, is recommended.

MPXV may be detected in semen, although evidence is still emerging about the diagnostic yield and clinical value of this specimen type [33].

3.3 Specimen collection

For details on specimen collection, handling and transport for MPXV testing, please refer to the 'PHLN Guidance on mpox patient referral, specimen collection and test requesting' [34]. Lesion samples should be collected on the tip of the swab and ideally exudate should be visible. Vigorously rub the bottom of the lesion to ensure cellular material from the lesion base is collected. It may be necessary to deroof the lesion. It is not recommended to use a scalpel for this due to the risk of sharps injury.

3.4 Specimen handling

Each sample should be placed in individual sterile containers or collection tubes. Avoid adding transport media as this may dilute the sample and increase the risk of leakage during transport. However, swabs that have already been placed into suitable transport medium (e.g. viral transport media) may be tested.

Specimen primary containers should be wiped down using a suitable detergent, followed by a Therapeutic Goods Administration (TGA) approved hospital-grade disinfectant with activity against viruses. This will be recorded on the label and product information. Alternatively, use a bleach solution. You can also use a TGA-listed 2-in-1 (single step) combined cleaning and disinfection product with activity against viruses. For more information, please refer to the TGA website for a list of suitable hospital-grade disinfectants [35]. Suitable disinfectants are also available from the United States Environmental Protection Agency [36].

Make sure specimen containers and tubes that contain fluid are screwed shut securely to prevent leakage during transport and place in a specimen bag. The specimen bag should contain sufficient absorbent material (for example, cotton wool or tissue), to absorb the entire contents of the primary receptacle in case there is any leakage. Place the specimen bag into a secondary container, such as a second specimen bag or rigid container prior to transport to the laboratory. Keep mpox specimens separate from other specimens.

After the specimen collection and packaging, conduct environmental cleaning as per the jurisdictional infection prevention and control advice for healthcare workers.

3.4.1 Personal protective equipment

Appropriate personal protective equipment (PPE) should be worn while collecting samples from patients with suspected mpox [34]. This includes disposable fluid resistant gown, disposable gloves, face shield or goggles, and a fluid-repellent surgical mask (FRSM). Consider wearing a P2/N95 PFR or equivalent if: the patient has respiratory symptoms;

varicella is suspected; or if there are other high-risk exposure events, for example prolonged exposure with the patient (such as hospitalised patient) or aerosol generating activities.

3.5 Specimen transport guidelines

For details on specimen transportation, refer to the 'Requirements for the packaging and transport of pathology specimens and associated materials (Fifth Edition 2022)' and 'Recommendations on the Transport of Dangerous Goods Volume I (Twenty-third revised edition 2023)'. Note that except for MPXV cultures, samples may be transported as category B biological hazards (UN 3373). MPXV cultures should be transported as category A biological hazards (UN 2814).

Submit specimens to the testing laboratory as soon as possible. If there is a delay in transport to the laboratory, refrigerate specimens (approximately 4 °C) or frozen (–20 °C or lower). You can store refrigerated specimens for up to 7 days, and frozen specimens for up to a month. Keep the specimens refrigerated during transport to the reference laboratory.

Raise any questions about specimen collection and transport with the specialist microbiologist to whom the specimen is being referred.

3.5.1 Laboratory handling of specimens and prevention of laboratory acquired infection

There is a risk of laboratory-acquired MPXV infection, but with appropriate biosafety this risk is low. Local risk-assessments should be conducted, and are the responsibility of each laboratory. There have been no reported cases of laboratory-acquired MPXV infection. Several cases, however, have been reported of laboratory-acquired Orthopoxvirus infections (largely vaccinia) as a result of needlestick or splash injuries [37–42]. Guidelines for the handling of laboratory specimens may change as further evidence for the risk of transmission in this setting is generated.

Vaccinia vaccination likely provides protection against MPXV infection and is recommended for laboratory workers in some settings [43-45]. Vaccinia vaccination is protective in animal models and may also have a role in post-exposure prophylaxis in cases of occupational exposure. Consideration of this should be included in local risk assessments depending on diagnostic sample burden, as risks of vaccinia vaccination may not outweigh benefit in low burden settings.

3.5.2 Microbiology specimens suspected to contain monkeypox virus

When handling clinical specimens suspected to contain MPXV, PPE should be worn according to appropriately conducted risk assessments undertaken by local laboratory management..

Diagnostic testing for MPXV from primary specimens should be conducted in a Physical Containment 2 (PC2) laboratory with appropriate PPE. Samples should be handled in a class II biological safety cabinet, particularly if any aerosol generating procedures are performed (e.g. vortexing or sonication). Sealed centrifuge rotors or sample cups should be used where available. If these specimens are also processed in bacteriology laboratories, after the plate and slide preparation, extra PPE precautions are not necessary.

Laboratory-based NAA testing for primary specimens suspected or confirmed to contain MPXV are handled at a different level of risk compared to MPXV cultures. If there is doubt about the associated level of risk, or any other questions about transport requirements, discuss these cases with the specialist microbiologist.

3.5.3 Routine chemistry, haematology and urinalysis testing

Standard laboratory precautions should be taken when processing samples from patients with suspected mpox. Vaccination of laboratory personnel handling these specimens is not recommended, in line with international guidance [43,44]. All laboratories handling monkeypox virus specimens should have completed an individual risk assessment for these processes. Precautions for handling of other non-microbiology specimen types should be considered by laboratories as part of their individual risk assessment.

3.5.4 Culture of monkeypox virus

Culture of MPXV should only be performed at specialised reference laboratories under PC level 3 or 4. Unless contraindicated, it is recommended that staff performing MPXV culture be vaccinated with vaccinia.

3.6 Access to diagnostic testing

MPXV testing is generally performed at jurisdictional PHLN laboratories. Prior to testing, suspected cases should be notified through the relevant state or territory Public Health Unit. Direct contact with the specialist microbiologist at the testing laboratory is essential to arrange receipt of specimens and obtain advice on specimen collection, safe packaging, and

transport. Excessive sample collection should be discouraged to minimise risk to healthcare workers or laboratory personnel.

3.7 Quality assurance programs

The Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) Biosecurity program offers a MPXV proficiency testing panel for PHLN member laboratories.

4 SNOMED CT terms

SNOMED CT code	Term name	Description
359814004	Monkeypox	*Disorder*
59774002	Monkeypox virus	*Organism*
1290831000000108	Monkeypox virus detection result positive	*Finding*

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6 Glossary

ARTG – Australian Register of Therapeutic Goods

DNA – Deoxyribonucleic acid

EDTA - Ethylenediaminetetraacetic acid

GISAID – Global Initiative on Sharing All Influenza Data

IgG – Immunoglobulin G

IgM – Immunoglobulin M

MPXV – Monkeypox virus

MSM – Men who have sex with men

MVA-BN – Modified Vaccinia Ankara-Bavaria Nordic

NAA – Nucleic acid amplification

NAAT – Nucliec acid amplification test

PC2 – Physical Containment Level 2

PC3 – Physical Containment Level 3

PHLN – Public Health Laboratory Network

PPE – Personal protective equipment

RCPAQAP – Royal College of Pathologists of Australasia Quality Assurance Program

TGA – Therapeutic Goods Administration

WHO – World Health Organization