



## 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
1.4	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

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## 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 from clinical specimens in the absence of exposure to another orthopoxvirus

### 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

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Monkeypox virus 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]. 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 which can infect a range of mammalian species (including humans and non-human primates), but the natural reservoir remains unknown[3]. Most cases have been reported in the Democratic Republic of the Congo 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[4–6]. In 2022, a large number of mpox cases 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)[7,8], prompting concern about potential to spread to other non-endemic countries, including Australia.

Monkeypox virus infection 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.[3,9].

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. The evolution of lesions progresses through four stages — macular, papular, vesicular, to pustular — before scabbing over and then healing.



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In the current outbreak, some features of the illness are atypical; anogenital and mucosal lesions are common, some people have had a localised rash without prodromal symptoms and a small number of cases have presented with anorectal pain without other clinical features. Care is largely supportive. The case fatality rate in mpox patients is estimated to be between 0–11% [10]. However, there are significant challenges in accurately estimating this rate[3]. Case fatality rates are lower for patients infected with Clade II compared to Clade I. 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[11–14]. 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 monkeypox[15,16]. In the US, (MVA-BN) JYNNEOS®, a newer vaccinia-based, live attenuated vaccine was specifically licensed for the prevention of monkeypox[17], 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 monkeypox virus infection.

## 3 Laboratory diagnosis

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### 3.1 Test method

#### 3.1.1 Nucleic acid amplification tests

Multiple in-house NAA assays have been described for detection of Orthopoxviruses including monkeypox virus using both gel-based and real time methods[1]. Assays using commercial platforms for the specific detection of monkeypox virus have been described[18,19]. At the time of writing one commercially supplied test kit for the detection of monkeypox virus 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 these assays show comparable limits of detection[20]. Multiplex NAA assays which differentiate Clades I and II of the monkeypox virus have been described [21], based on fragment length of the NAA product of the E5R gene. As the analytical sensitivity of NAA assays used in the detection of orthopox viruses is high[22], they may potentially be used to exclude a diagnosis of monkeypox virus infection from an appropriately collected sample. Where Orthopoxvirus DNA is detected, monkeypox virus-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 monitoring outbreaks and investigation of virus introductions from animal reservoirs into the human population as well as human-to-human transmission events[23]. Both Illumina and Oxford Nanopore sequencing technologies have been used for genome sequencing[24].

#### 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. Orthopox viruses have a distinct brick-shaped morphology when examined with sodium phosphotungstate or other heavy-metal negative stains.

### 3.1.4 Virus growth assays

Orthopox viruses can be isolated in a variety of cell lines including Vero, Buffalo Green Monkey and HeLa cells. Subculture to chick chorioallantoic membrane cells has been the method of identification, but newer methods such as NAA are increasingly used for end-point determination. The sensitivity and specificity of this method are uncertain, and it is performed at reference laboratories only under PC3 conditions.

### 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 epidemiologic surveillance studies, but its utility for the diagnosis of acute monkeypox virus infection is uncertain. Testing for monkeypox virus-specific IgM can be considered within five days of presentation, or IgG after eight days[25]. An IgM capture assay[26] 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 monkeypox virus may be cross-reactive with other orthopoxviruses, including Vaccinia virus-based vaccines. 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 monkeypox virus serology.

## 3.2 Suitable specimen types

Lesion material should be collected from persons with suspected monkeypox virus 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 nucleic acid amplification (NAA) testing.



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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 monkeypox virus, but are often negative due to the transient nature of viraemia[27], and therefore should not be used to exclude infection with monkeypox virus. A minimum of 5 mL of EDTA whole blood or 10 mL of serum is recommended.

Monkeypox virus may be detected in semen, although evidence is still emerging about the diagnostic yield and clinical value of this specimen type[28].

### 3.3 Specimen collection

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 derroof the lesion. If there is no obvious lesion, for example, macular rash alone, discuss approaches with a specialist microbiologist. For patients presenting with proctitis and no visible lesion, insert a swab to sample the anorectal mucosa, avoiding excess faeces contamination. 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 also be tested.

### 3.4 Specimen handling

#### 3.4.1 Laboratory handling of specimens and prevention of laboratory acquired infection

There is a risk of laboratory-acquired monkeypox virus 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 monkeypox virus infection. Several cases, however, have been reported of laboratory-acquired Orthopoxvirus infections (largely vaccinia) as a result of needlestick or splash injuries[29–34].

Vaccinia vaccination likely provides protection against monkeypox virus infection, and is recommended for laboratory workers in some settings [35–37]. Vaccinia vaccination may also have a role in post-exposure prophylaxis in cases of occupational exposure [35]. 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.

Guidelines for the handling of laboratory specimens may change as further evidence for the risk of transmission in this setting is generated.

### 3.4.2 Personal protective equipment

Appropriate personal protective equipment (PPE) should be worn while collecting samples from patients suspected of monkeypox virus infection [38]. 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
- There are other high-risk exposure events, for example prolonged exposure with the patient (such as hospitalised patient) or other aerosol generating activities.

Specimens 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<sup>1</sup>. Suitable disinfectants are also

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<sup>1</sup> <https://www.tga.gov.au/disinfectants-sterilants-and-sanitary-products>





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available from the United States Environmental Protection Agency website<sup>2</sup>. After the specimen collection, conduct environmental cleaning as per the Infection Prevention and Control Expert Group (ICEG) interim guidance on mpox for health workers advice<sup>3</sup>.

Diagnostic testing for monkeypox virus should be conducted in a Physical Containment 2 (PC2) laboratory, provided appropriate PPE is used, guided by an appropriately conducted risk assessment undertaken by local laboratory management. If these specimens are also processed in bacteriology laboratories, after the plate and slide preparation, extra PPE precautions are not necessary. Handling of monkeypox virus isolated from clinical samples should be at PC3, at a minimum.

### 3.4.3 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 guidance from the United States Centers for Disease Control and Prevention (US CDC)[39]. 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.

### 3.4.4 Microbiology specimens suspected to contain monkeypox virus

When handling clinical specimens suspected to contain monkeypox virus, PPE should be worn according to appropriately conducted risk assessments undertaken by local laboratory management. For additional guidance, refer to the ICEG interim guidance on mpox for health workers advice<sup>4</sup>.

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<sup>2</sup> <https://www.epa.gov/pesticide-registration/disinfectants-emerging-viral-pathogens-evps-list-q>

<sup>3</sup> <https://www.health.gov.au/resources/publications/iceg-interim-guidance-on-monkeypox-for-health-workers>

<sup>4</sup> <https://www.health.gov.au/resources/publications/iceg-interim-guidance-on-monkeypox-for-health-workers>



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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.

### 3.4.5 Culture of monkeypox virus

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

## 3.5 Specimen transport guidelines

Samples which are suspected to contain monkeypox virus are handled at a different level of risk as confirmed samples. Samples which are suspected to contain monkeypox virus may be considered as category B infectious substances. Please note however, that those without proven mpox but with a very high pre-test probability (e.g. symptomatic close contact of a known case, or clinical samples where Orthopoxvirus DNA has been detected) represent an increased risk and may be considered as category A substance for the purposes of transport. If there is doubt as to the associated level of risk, these cases should be discussed with the local public health laboratory prior to transportation.

Following collection, the primary receptacle should be placed in a specimen bag, followed by another secondary specimen bag prior to transport. Specimen containers and tubes that contain fluid should be screwed shut securely to prevent leakage during transport. For non-solid samples, the primary receptacle should be placed in a specimen bag with sufficient absorbent material (e.g. cotton wool or tissue) to absorb the entire contents of the primary receptacle. This should then be placed in another secondary container as per above prior to transport. For additional details on specimen packaging and transportation, refer to [“Requirements for the Packaging and Transport of Pathology Specimens and Associated Materials Fourth Edition 2013”](https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-mpaac-docs-PackTransPathSpecimens.htm) available at: (<https://www1.health.gov.au/internet/main/publishing.nsf/Content/health-mpaac-docs-PackTransPathSpecimens.htm>)

Samples should be submitted to the testing laboratory as soon as possible. If there is a delay in transport to the laboratory, samples should be stored refrigerated or frozen at  $-20^{\circ}\text{C}$  or

lower. Refrigerated samples can be stored for up to 7 days, and frozen samples for up to a month[40]. During transport to the reference laboratory samples should be kept refrigerated.

### 3.6 Access to diagnostic testing

Testing is performed at jurisdictional PHLN laboratories. Prior to testing, suspected cases must be notified through the relevant state or territory Public Health Unit. Direct contact with the medical 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 monkeypox proficiency testing panel for PHLN member laboratories.

## 4 SNOMED CT terms

SNOMED CT code	Term name	Description
<b>359814004</b>	Monkeypox	<i>Disorder</i>
<b>59774002</b>	Monkeypox virus	<i>Organism</i>
<b>1290831000000108</b>	Monkeypox virus detection result positive	<i>Finding</i>



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