

# Laboratory testing for the monkeypox virus

Interim guidance

23 May 2022



## Key points

- The goal of the global response to the multi-country outbreak of monkeypox is to stop the outbreak.
- Any individual that meets the suspected case definition for monkeypox should be offered testing.
- The recommended specimen type for diagnostic confirmation of monkeypox in suspected cases is skin lesion material, including swabs of lesion exudate, roofs from more than one lesion, or lesion crusts.
- Laboratory confirmation of specimens from a suspected case is done using nucleic acid amplification testing (NAAT), such as real-time or conventional polymerase chain reaction (PCR). NAAT can be generic to *orthopoxvirus* (OPXV) or specific to *monkeypoxvirus* (MPXV, preferable).
- All manipulations in laboratory settings of specimens originating from suspected, probable or confirmed cases of monkeypox should be conducted according to a risk-based approach.
- In addition to NAAT, sequencing is useful to determine virus clade and to understand epidemiology. Member States are strongly encouraged to share MPXV genetic sequence data in available and publicly accessible databases.
- Member States are requested to immediately notify WHO under the International Health Regulations (IHR) 2005 of positive laboratory results, including a generic OPXV laboratory test that awaits confirmation.
- WHO can assist Member States to access testing through referral. If the need arises, Member States can contact the relevant WHO Regional Office.

## Introduction

*Monkeypoxvirus* (MPXV) is a double-stranded DNA virus, a member of the *orthopoxvirus* genus within the *Poxviridae* family. Poxviruses cause disease in humans and many other animals; infection typically results in the formation of lesions, skin nodules or disseminated rash. Other *orthopoxvirus* (OPXV) species pathogenic to humans include *cowpox virus*, and *variola virus* (causing smallpox, which has been eradicated). *Vaccinia virus* is also an OPXV that has been used to vaccinate people and was a key tool for the eradication of smallpox, achieved in 1980. MPXV is named due to its initial detection in monkeys. MPXV can primarily be found in rodents, however the reservoir is undetermined. There are two known clades of MPXV, one endemic in Western Africa and one in the Congo Basin region.

The typical presentation of monkeypox is well described and consists of a short febrile prodromal period followed by progressive development of a classic rash with indurated and umbilicated (centrally depressed) lesions, starting on the head or face and progressing to the limbs and trunk. Lesions progress all at the same stage from macules, to papules, to vesicles, to pustules and eventually to crusts which dry up and fall off after two to four weeks. There are often enanthem (sores or ulcers) in the mouth and lesions can affect the eyes and/or genital area. Swollen lymph nodes are typical of monkeypox. However, lesions may be haemorrhagic or coalesce into large bullae. In this multi-country outbreak, there have been suggestions that the progression of the lesions may be atypical, beginning in the genital area. Many persons experiencing this condition may have been tested for other infectious diseases at the time of detection.

This guidance serves to provide interim recommendations to laboratories and stakeholders involved in the diagnosis of monkeypox. These recommendations have been prepared by WHO in consultation with and reviewed by subject matter laboratory experts, with experience handling and detecting MPXV and OPXV, and those with expertise in the development of diagnostic assays for OPXV. For countries that have regulatory standards that apply to clinical laboratory testing performed on human specimens, those regulatory standards should appropriately be followed. WHO is closely monitoring developments related to this outbreak and will revise and publish updated recommendations as necessary. Unless revisions are made, this document will expire in one year (May 2023).

## Indications for testing

Any individual meeting the definition for a suspected case should be offered testing.(1) The decision to test should be based on both clinical and epidemiological factors, linked to an assessment of the likelihood of infection. Due to the range of conditions that cause skin rashes and because clinical presentation may more often be atypical in this outbreak, it can be challenging to differentiate monkeypox solely based on the clinical presentation, particularly for cases with an atypical presentation. It is therefore important to consider other potential causes of discrete skin lesions or a disseminated rash; Examples of other aetiologies for similar-appearing skin lesions at the different stages of development include herpes simplex virus, varicella zoster virus, molluscum contagiosum virus, enterovirus, measles, scabies, *Treponema pallidum* (syphilis), bacterial skin infections, medication allergies, parapoxviruses (causing orf and related conditions) and chancroid (2).

## Specimen collection, shipment and storage

**Safety procedures.** Use of adequate standard operating procedures (SOPs) must be ensured and laboratory personnel must be trained for appropriate donning and doffing of personal protective equipment (PPE), specimen collection, storage, packaging and transport. All specimens collected for laboratory investigations should be regarded as potentially infectious and handled with caution. Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing routine clinical specimens from confirmed or suspected monkeypox patients. These may include limiting the number of staff testing specimens only to staff with proven competency, wearing appropriate PPE, using rigorously applied standard precautions, and avoiding any procedures that could generate infectious aerosols. Where appropriate and available, consideration of vaccination among staff is encouraged. Effective disinfectants include quaternary ammonium compounds and 0.5% (or 200ppm) bleach (freshly made).

Rigorous adherence to infection prevention and control guidelines must be ensured during specimen collection and handling (Clinical management and IPC guidance is in development)

**Specimen to be collected.** The recommended specimen type for laboratory confirmation of monkeypox is skin lesion material, including swabs of lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts. Swab the lesion vigorously, to ensure adequate viral DNA is collected. Both dry swabs and swabs placed in viral transport media (VTM) can be used. Two lesions of the same type should be collected in one single tube, preferably from different locations on the body and which differ in appearance. Lesions, crusts and vesicular fluids should not be mixed in the same tube. If resources permit it, two tubes may be collected to minimise risk of poor sampling or inhibitors, however only one should be tested and the second should only be tested in case the first provides inconclusive results. In addition to a lesion specimen, the collection of an oropharyngeal swab is encouraged. However, data on the accuracy of this specimen type for diagnosis is limited for monkeypox, therefore a negative throat swab specimen should be interpreted with caution.

Because the current outbreak is still under investigation, collection of additional specimen types for research purposes can be considered if allowed by the appropriate ethical review board, and there is sufficient laboratory and medical expertise for their safe collection, handling, and storage. These may include urine, semen, rectal and/or genital swab on indication based on clinical presentation including location of lesions. EDTA blood may support detection of MPXV but may not contain the high level of virus found in lesion samples, as any viremia occurs early in the course of infection, usually in the prodromal period, and before skin lesions become manifest. Collection of a lesion biopsy during the macular stage should be considered only if clinically indicated and only be performed by personnel with appropriate training. These additional specimen types are not intended for routine diagnostic purposes and do not need to be collected outside of research settings. More details on specimen collection and storage are included in the Annex.

Antibody detection from plasma or serum should not be used alone for diagnosis of monkeypox. However, IgM detection from recent acutely ill patients or IgG in paired serum samples, collected at least 21 days apart, with the first being collected during the first week of illness, can aid diagnosis if tested samples yield inconclusive results. Recent vaccination may interfere with serological testing.

**Packaging and shipment of clinical specimens.** Specimens should be stored refrigerated or frozen within an hour of collection and transported to the laboratory as soon as possible after collection. Correct handling and storage of specimens during transportation is essential for accurate diagnostic testing (see Annex). Transport of specimens should comply with any applicable national and/or international regulations, including the UN Model Regulations and any other applicable regulations depending on the mode of transport being used. For international transport, specimens from suspected probable or confirmed cases of MPXV, including clinical samples, viral isolates and cultures should be transported as Category A, UN2814 “infectious substance, affecting humans.” All specimens being transported should have appropriate triple packaging, labelling and documentation. Shipping requires a dangerous goods certified shipper. For information on infectious substances shipping requirements, please see the WHO Guidance on regulations for the transport of infectious substances 2021-2022 (3).

**Specimen storage.** Specimens collected for MPXV investigation should be refrigerated (2 to 8°C) or frozen (-20°C or lower) within one hour after collection. If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20°C or lower. Longer term specimen storage (>60 days from collection) is recommended at -70°C. Viral DNA present in skin lesion material is relatively stable if kept in a dark, cool environment, which can be considered when cold chain is not readily available (4), but room temperature shipment is not recommended until further studies provide evidence that sample quality is not compromised. Repeated freeze-thaw cycles should be avoided because they can reduce the quality of specimens. Aside from specific collection materials indicated in the annex, other requisite materials and equipment may include: transport containers and specimen collection bags and triple packaging, coolers and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, and materials for decontamination of surfaces.

## Laboratory testing methods and algorithm

Testing for the presence of MPXV should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. Confirmation of MPXV infection is based on nucleic acid amplification testing (NAAT), using real-time or conventional polymerase chain reaction (PCR), for detection of unique sequences of viral DNA. PCR can be used alone, or in combination with sequencing. Several groups have developed validated PCR protocols for the detection of OPXV and more specifically MPXV, some of which include distinction of Congo Basin and West African clades (5–9). Some protocols involve two steps, in which the first PCR reaction detects OPXV, but does not identify which species. This can then be followed by a second step, which can be PCR-based or utilize sequencing, to specifically detect MPXV. Before an assay is utilized to test human clinical specimens within a laboratory, it should be validated and/or verified within the laboratory by appropriately trained staff.

**Reagents.** Reagents should be stored according to manufacturer recommendations. There are a number of primer and probe sequence sets for PCR assays for OPXV and specifically MPXV that have been published in the literature and can be used for in house development of assays in laboratories with appropriate capacities (5–7). PCR kits detecting OPXV or specifically MPXV are under development (10,11), but no commercial PCR or serology kits are currently widely available. Positive control material for PCR assays can be ordered from specialized initiatives (12). Best practice is that the positive control should be included at a low (above the limit of detection) but easily detectable concentration; inclusion of quality control materials where possible can assist in controlling for any assay issues. Controls should provide information about (1) sample quality, (2) nucleic acid quality, and (3) process quality. PCR can be extremely sensitive so efforts should be made to limit contamination, and negative controls on every run should be utilized to ensure contamination has not occurred. Sample integrity controls (e.g. RNase P), extraction, positive and inhibition controls can help in distinguishing a false negative from a true negative. Controls should be utilized following laboratory SOPs. If any of the assay controls fail, testing should be repeated.

**Disposal of Waste.** All waste that may contain MPXV should be decontaminated before disposal by using an approved method, such as autoclaving or chemical disinfection as approved by the specific laboratory procedures.

**Electron microscopy.** Electron microscopy can be used to evaluate the sample for a potential poxvirus, but with the availability of molecular assays and the high technical skills and facility required, this method is not routinely used for the diagnosis of poxviruses.

**Viral culture.** Virus isolation is not recommended as a routine diagnostic procedure and should only be performed in laboratories with appropriate experience and containment facilities. As these methods are not recommended as part of routine diagnosis, the specific details for these methodologies are not covered in this document.

## Interpretation of laboratory results

Confirmation of MPXV infection should consider clinical and epidemiological information. Positive detection using an OPXV PCR assay followed by confirmation of MPXV via PCR and/or sequencing, or positive detection using MPXV PCR assay in suspected cases in endemic and non-endemic areas indicates confirmation of MPXV infection. While it is preferable to perform MPXV specific confirmatory testing, positive detection using OPXV PCR assay is considered sufficient for laboratory confirmation of suspected cases in non-endemic countries. Member States are requested to immediately notify WHO of laboratory confirmed cases.

When the clinical presentation and epidemiology suggest an infection with MPXV despite negative PCR results, serological testing may be useful to further investigate prior infection for epidemiological purposes. A number of factors could contribute to false-negative results, such as poor quality of specimen, wrong handling or shipping, or technical reasons inherent to the test, e.g. DNA extraction failure.

In addition to the use of sequencing for diagnosis, genetic sequence data (GSD) may also provide valuable information to help understand the origins, epidemiology and characteristics of the virus, for example whether cases arise from a single introduction or multiple introductions from other locations. Sequencing of MPXV from as many positive specimens from different patients as possible, is recommended at this stage. WHO strongly encourages countries and laboratories to share GSD, including raw data whenever possible in a timely manner through the available public access databases. GSD can be generated using Sanger or next generation sequencing (NGS) methods.

## Biological risk management

It is recommended that all manipulations of specimens originating from suspected, probable or confirmed cases of monkeypox in the laboratory be conducted according to a risk-based approach. Each laboratory should conduct a local (that is, institutional) risk assessment. When manipulating biological specimens, core biosafety requirements, similar to those previously referred to as biosafety level 2, must be met and heightened control measures should be applied based on local risk assessment.

MPXV may be contracted during the specimen processing stage from contaminated material or faulty processes. Therefore, heightened biosafety measures are recommended in addition to the core requirements, including the following for the purpose of clinical testing without virus propagation:

- Specimens from patients with suspected MPXV infection must be handled in a functioning Class I or II biosafety cabinet, prior to sample inactivation. Properly inactivated specimens do not require a biosafety cabinet.
- Laboratory personnel should wear appropriate PPE, especially for handling specimens before inactivation.
- Where use of a centrifuge is required for a procedure, safety cups or sealed rotors should be used.

Additional control measures should be considered for specific procedures, including aerosol-forming procedures, according to the local risk assessment. For more information on core biosafety requirements and heightened control measures, please see the fourth edition of the WHO Biosafety Manual (13).

## Occupational health

Various smallpox vaccines, containing vaccinia virus, provide cross-protection against other OPXV, including monkeypox, therefore national health authorities should conduct a risk assessment and consider whether arranging immunization for health care workers, including laboratory personnel, and other staff that are at risk of exposure to individuals or specimens with MPXV is required. (14). A non-replicating vaccine consisting of the modified vaccinia Ankara strain known as MVA-BN was approved for prevention of smallpox (which was declared eradicated in 1980) in 2013. In

2019 it was also approved for the prevention of monkeypox by two stringent regulatory authorities. This vaccine can also be considered for prevention of monkeypox in the occupational setting. One antiviral therapeutic has been approved for the treatment of smallpox and monkeypox. Guidance on immunization and treatment will be provided separately.

## Reporting of cases and test results

Laboratories should follow national reporting requirements. All test results, positive or negative, should be immediately reported to national authorities. States Parties to the IHR are reminded of their obligations to share with WHO relevant public health information for events for which they notified WHO, using the decision instrument in Annex 1 of the IHR (2005) (15).

## Global laboratory networking

Access to timely and accurate laboratory testing of samples from cases under investigation is an essential part of the diagnosis and surveillance of this emerging infection. All countries should have access to reliable testing either nationally or through referral to laboratories in other countries that are willing and able to perform OPXV or MPXV diagnosis. WHO, through its Regional Offices, can assist Member States to access testing through referral. Where appropriate and safely performed, inactivation of samples in the local laboratory may facilitate referral and ease logistical challenges. Countries are encouraged to share their sequence data for a better understanding of the current outbreak. The US Centers for Disease Control and Prevention is the WHO Collaborating Centre for Smallpox and Other Poxvirus Infections (United States of America) and the Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology, "VECTOR" (Russian Federation) is the WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA.

## Process and Methodology

This document was developed in consultation with global external experts in the field of OPXV and other laboratory expertise. WHO staff across the organization with expertise in laboratory diagnosis, epidemiology and clinical management were also consulted. This version of the guidance incorporates the latest understanding and characteristics of the virus and addresses questions and issues received from WHO's country and regional offices and other channels.

## Limitations

This guidance serves to provide interim recommendations for the diagnosis of MPXV infection in the context of the current multi-country outbreak in non-endemic countries in multiple regions of the world (May 2022). WHO will issue further updates to this interim guidance as necessary. This guidance will be updated as more specific information about the epidemiology of this outbreak becomes available.

## Plans for updating

WHO work with experts around the world and continues to monitor the situation closely for any changes that may affect this interim guidance. WHO will issue a further update. Otherwise, this interim guidance will expire one year after the date of publication.

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## Declaration of interests

Experts in the network completed a confidentiality agreement and declaration of interest. The declaration of interest forms were reviewed, and no conflicts regarding the support of this guidance document were identified.

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## Annex. Specimen collection and storage

Specimen type	Collection materials	Storage temperature	Collection purpose
Skin lesion material, including: <ul style="list-style-type: none"> <li>– Swabs of lesion exudate</li> <li>– Lesion roofs</li> <li>– Lesion crusts</li> </ul>	Dacron or polyester flocked swabs with VTM or dry swab	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	Recommended for diagnosis
Oropharyngeal swab	Dacron or polyester flocked swabs with VTM or dry swab	See above	Recommended for diagnosis if feasible, in addition to skin lesion material
Rectal and or genital swabs	Dacron or polyester flocked swabs with VTM or dry swab	See above	To be considered for research (following ethics guidelines)
Urine	Sterile collection tube	See above	To be considered for research (following ethics guidelines)
Semen	Sterile collection tube	Room temperature for <1h (then -20°C or lower)	To be considered for research (following ethics guidelines)
Whole blood	Sterile collection tube with EDTA	See above	To be considered for research (following ethics guidelines)
Serum	Serum-separating tubes	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	To be considered for serology to aid diagnosis or research (following ethics guidelines)
Plasma	collection tube with EDTA	See above	To be considered for serology to aid diagnosis or research (following ethics guidelines)

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