Diagnostic testing for the monkeypox virus (MPXV)

Interim guidance

10 May 2024

World Health Organization

Key points

- Any individual meeting the case definitions for suspected or probable mpox should be offered testing.(1)
- Testing for the presence of MPXV should be performed in appropriately equipped laboratories by staff trained in relevant technical and safety procedures and conducted under relevant biosafety conditions based on a risk-based approach.
- The recommended specimen type for diagnostic confirmation of monkeypox virus (MPXV) infection in suspected cases is lesion material.
- Alternative specimen types, such as oropharyngeal swabs, can be collected from individuals who are contacts of suspected or confirmed mpox cases but have no visible skin or mucosal lesions. Note that these may lack sensitivity in pre-symptomatic cases, and testing should be repeated on lesion material if rash or mucosal disease develops.
- The presence of virus is confirmed by nucleic acid amplification testing (NAAT), such as real-time or conventional polymerase chain reaction (PCR). It is important for assays to target conserved orthopoxvirus (OPXV) or MPXV genes, to minimize the risk of assays being affected by sequence variants or gene dropouts.
- MPXV-clade specific NAAT and/or sequencing facilitates interpretation of mpox disease epidemiology. Scientists and public health professionals are strongly encouraged to share MPXV genetic sequence data in available and publicly accessible databases.
- WHO has released target product profiles for tests to be used for mpox diagnosis, highlighting key targets for test developers to pursue to optimize public health benefit and impact.(2)
- This document provides interim guidance for clinicians, laboratories, health workers, public health officials and other stakeholders involved in the diagnosis and care of patients with suspected, probable or confirmed mpox.
- This version of the interim guidance has been updated to reflect developments in mpox epidemiology and viral evolution with respect to the emergence of strains of Clade I MPXV with mutations that may evade diagnostic confirmation depending on the protocol targets.
- This is an updated version of the interim guidance on *Diagnostic testing for the monkeypox virus (MPXV)* and supersedes the guidance published on 9 November 2023.

Changes from earlier version

This is an updated version of the interim guidance on *Diagnostic testing for the monkeypox virus* and supersedes the guidance published on 9 November 2023. This version includes updated recommendations to highlight diagnostic strategies to avoid gene target failures and determine monkeypox virus (MPXV) clades. This version is in alignment with other updated interim guidance documents published by WHO since November 2023.

Introduction

Mpox (formerly known as monkeypox) is an infectious disease caused by the monkeypox virus (MPXV), a double-stranded DNA virus, that belongs to the *Orthopoxvirus* genus of the *Poxviridae* family. The virus was first discovered in 1958 as the cause of outbreaks of a pox-like disease in monkeys kept for research in Denmark. Human disease was first identified in 1970 in a 9-month-old boy in the Democratic Republic of the Congo.(3–5) Orthopoxviruses can cause disease in humans and other mammals. Symptomatic infection typically results in the formation of lesions, skin nodules or disseminated rash. Other orthopoxviruses (OPXVs) pathogenic to humans include *Cowpox virus* and *Variola virus (*causing smallpox). Smallpox vaccines derived from *Vaccinia virus*, also an orthopoxvirus, were a key tool for the eradication of smallpox, which was achieved in 1980.

MPXV was named based on its initial detection in captive monkeys and, although the virus continues to affect species of monkeys in Africa, the main animal reservoirs are likely small forest mammals such as rodents and squirrels. There are two known clades of MPXV: clade I, mainly found in the Congo Basin region, and clade II, which contains two subclades, designated as clade IIa and clade IIb. (6) Clade IIb is the predominant strain in the ongoing global outbreak, which was recognized in May 2022 and has disproportionally affected men who have sex with men. Over many years and especially since 2012, an increasing trend in clade I detections has also been reported in the Democratic Republic of the Congo with a marked rise in cases reported since 2023. In addition to transmission in endemic areas considered to be often zoonotic transmission with associated household and community spread (7), human-to-human transmission of mpox due to clade I MPXV has also continued to increase. For the first time in 2023, sexual transmission of clade I MPXV has also been documented in the Democratic Republic of the Congo, where outbreaks associated with sexual contact have occurred and are ongoing (8,9). Outbreaks linked to sexual transmission are occurring among sex workers in mining communities, in clusters of cases among men who have sex with men, and through heterosexual transmission in households.

MPXV has a linear DNA genome and is approximately 200 kb long. The genome has a highly conserved central region coding for replication and its assembly machinery. (10)In addition, the genome has the more variable ends that contain inverted terminal repeats (ITRs) ends, which contain genes involved in host range determination and pathogenesis. MPXV contains at least 4 open reading frames (ORFs) in the ITR region (11). For clade I and clade II, deletions and duplications of parts of the less conserved regions in the MPXV genome termini have been described (11–17). These deletions can lead to loss of detection with MPXV clade specific NAAT tests. (12,14,18) Therefore monitoring the genomic evolution of MPXV and the potential impact on performance of NAAT assays used is strongly recommended. NAAT assays are further discussed in the laboratory-based testing methods section.

Mpox can cause a range of signs and symptoms. The classic incubation period ranged from 5 to 21 days.(19) The classic mpox presentation is a short prodromal phase lasting 1-5 days. During this time patients may experience fever, headache, back pain, muscle aches, and lymphadenopathy.(20) The second phase typically occurs after the fever subsides, with the appearance of skin and/or mucosal rash, which might include a single or multiple lesions.(21,22) Typically, the lesions progress through macules, papules, vesicles, and pustules, before crusting over and desquamating over a period of 2 to 4 weeks.(3)

During the 2022-2023 multi-country mpox outbreak, the recorded incubation period ranged from 1 to 12 days (23), occasionally last much longer (up to 40 days).(25) Patients presented with more mucosal lesions than previously described, often localized in the genital or perineal/perianal area and / or involving the mouth and eyes.(26,27) The prodromal period was often absent: an observational study reported that in half of patients, skin lesions were the first sign of infection.(28) Anorectal pain and bleeding (e.g. due to proctitis) was also newly reported in the global outbreak.(1) Lymphadenopathy remains a common feature of mpox, usually appearing early in the course of illness.(26)

Persons with suspected, probable or confirmed mpox, should follow appropriate isolation and infection control guidance to prevent onward transmission. Similarly, health workers and laboratory personnel should also follow appropriate infection prevention and control (IPC) measures, including use of personal protective equipment (PPE),

to prevent infection. Detailed information and recommendations for clinical management, including treatment options and IPC strategies, can be found here.(29) These measures are advised until all skin lesions are epithelialized, crusts fall off, and other symptoms such as proctitis have subsided.

Point-of-care technologies are emerging, but currently mpox diagnosis is primarily reliant on laboratory-based nucleic acid amplification testing (NAAT). WHO has released target product profiles for NAAT assays to be used for mpox diagnosis within health care settings and laboratories and for tests targeting orthopoxvirus antigen(s) to be used as an aid to mpox diagnosis for decentralized use, including in the community.

Target audience

This document provides interim guidance for clinicians, laboratories, health workers, public health officials and other stakeholders involved in the diagnosis and care of patients with suspected or confirmed mpox.

Indications for testing

Any individual meeting the locally adapted WHO definition for a suspected or clinically compatible case of mpox should be offered testing (see Box 1). The decision to test should be based on both clinical and epidemiological factors, linked to an assessment of the likelihood of infection and the risk of further spread.

The rash that develops in mpox may resemble other infectious diseases or conditions, making it challenging to differentiate mpox solely based on clinical presentation. It is therefore important to consider other potential causes of discrete skin lesions or a disseminated rash; including varicella zoster virus (VZV, chickenpox), measles, scabies, herpes simplex virus (HSV), *Treponema pallidum* (syphilis); other OPXV in different settings such as buffalopox or bovine vaccinia or manifestations of vaccinia infection; parapoxviruses (causing orf or molluscum contagiosum), and rarely tanapox. Other causes of rash in the differential diagnosis may include disseminated gonococcal infection (DGI), vasculitis, bacterial skin and soft tissue infections, medication allergies and chancroid.(1,29,30)

Box 1: Definition of a suspected case (from "Surveillance, case investigation and contact tracing for mpox" (1)):

A person who is a contact of a probable or confirmed mpox case in the 21 days before the onset of signs or symptoms, and who presents with any of the following: acute onset of fever (>38.5°C), headache, myalgia (muscle pain/body aches), back pain, profound weakness or fatigue.

OR

A person presenting since 01 January 2022 with an unexplained acute skin rash, mucosal lesions or lymphadenopathy (swollen lymph nodes). The skin rash may include single or multiple lesions in the ano-genital region or elsewhere on the body. Mucosal lesions may include single or multiple oral, conjunctival, urethral, penile, vaginal, or anorectal lesions. Anorectal lesions can also manifest as anorectal inflammation (proctitis), pain and/or bleeding.

AND

for which the following common causes of acute rash or skin lesions do not fully explain the clinical picture: varicella zoster, herpes zoster, measles, herpes simplex, bacterial skin infections, disseminated gonococcal infection, primary or secondary syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, molluscum contagiosum, allergic reaction (e.g. to plants); and any other locally relevant common causes of papular or vesicular rash.

Prior infection with mpox or vaccination does not guarantee full protection from future infection. Therefore, if an individual presents with clinical symptoms suggestive of mpox, it is crucial that they seek prompt medical attention; undergo testing for mpox, HIV and other STIs as indicated particularly for cases that may be linked to sexual transmission; and receive appropriate medical care.(31) If resources are limited, patients at high-risk for severe infection such as immunocompromised individuals, should be prioritized for testing.

Currently, there are insufficient data on the usefulness or cost-effectiveness of screening for MPXV in asymptomatic individuals at high risk of infection.(32,33)

Specimen collection, shipment and storage

Safety procedures and preventive vaccination for personnel: 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), and 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 patients with suspected or confirmed mpox. 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. Use of sharp instruments should be avoided. It should also be noted that there may be high rates of undetected HIV in persons with suspected or confirmed mpox, which heightens the importance of universal precautions.(34,35)

The WHO Strategic Advisory Group of Experts on immunization (SAGE) recommends primary preventive vaccination (PPV) for persons at risk during outbreaks including children, as well as for health workers, including laboratory personnel, at risk for repeated exposure. SAGE also recommends post-exposure preventive (PEPV) vaccination for contacts of cases, ideally within 4 days of exposure (and up to 14 days in the absence of symptoms).(36,37)

Effective disinfectants include quaternary ammonium compounds and 0.5% bleach (freshly made). Rigorous adherence to infection prevention and control guidelines must be ensured during specimen collection and handling.(29)

Specimen to be collected (see Annex). The recommended specimen type for laboratory confirmation of MPXV is skin lesion material, including swabs of lesion surface and/or exudate, or lesion crusts. Swab the lesion vigorously, to ensure adequate viral DNA is collected. Swabs can be transported dry in capped tubes or placed in viral transport media (VTM). Specimens from two lesions should be collected in one single tube, preferably from different locations on the body. Lesions, crusts and vesicular fluids should not be mixed in the same tube. Because the definition of a suspected case includes symptomatic contacts of confirmed or probable mpox cases, alternative specimen types, such as oropharyngeal swabs, can be collected in the absence of skin or mucosal lesions. However, such specimen types provide less sensitive results for diagnosis than material from skin lesions. For this reason, a negative result should be interpreted with caution (38–42). Blood specimens are generally not useful for diagnosis of acute illness, unless this is taken to rule out other infections. The type of specimen may depend on the clinical presentation and contact exposure (see the Annex).

Collection of additional specimen types for research purposes can be considered if it is allowed by the appropriate ethics review board and there is sufficient laboratory and medical expertise for their safe collection, handling, and storage. These may include urine, semen, vitreous fluid or cerebrospinal fluid on indication and based on clinical presentation, including location of lesions and contact exposure.(26,39,40,43). EDTA blood may support detection of MPXV but may not contain the high level of virus found in lesion specimens because any viremia usually occurs early in the course of infection in the prodromal period before skin lesions become manifest and is of short duration. 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.

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 the Annex). Transport of specimens should comply with any applicable national and/or international regulations, including the UN Model Regulations 23rd edition(44) and any other applicable regulations depending on the mode of transport being used. For international transport, specimens from suspected, probable or confirmed mpox cases, excluding viral isolates and cultures– should be transported in principle as Category B, UN3373 “infectious substance, affecting humans”, unless national regulations specify otherwise. (45,46)

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 2023-2024.(45)

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 specimen 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 a cold chain is not readily available, (47) but room temperature shipment is not recommended until further studies provide evidence that specimen 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, specimen collection bags, 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-based testing methods

Testing for the presence of MPXV should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures.

Nucleic acid amplification testing.

WHO recommends confirmation of MPXV infection based on nucleic acid amplification testing (NAAT), using real-time or conventional polymerase chain reaction (PCR) on lesion material for detection of unique sequences of viral DNA. PCR can be used alone or in combination with sequencing for clade determination.

The selection of the initial NAAT and follow up procedure require adaptation to the local context. It is critical that the NAATs used are designed to detect highly conserved regions of the genome to reduce the risk of missing cases due to genomic deletions.(14,18) Highly conserved NAATs can consist of a generic OPXV or MPXV PCR, which can be followed by respectively MPXV specific or clade specific NAATs for positive samples for confirmation or epidemiological investigation. If clade specific NAATs fail to detect mpox, subsequent sequencing can be used for clade determination and genetic characterization. For a potential workflow see Figures 1 and 2.

Commercial assays should clearly articulate what clades can and cannot be detected: ideally, targets should be confidentially shared with regulatory authorities, and manufacturers should track potential target gene failure., and advise end users if their assay is affected by a known target dropouts.(9,14)

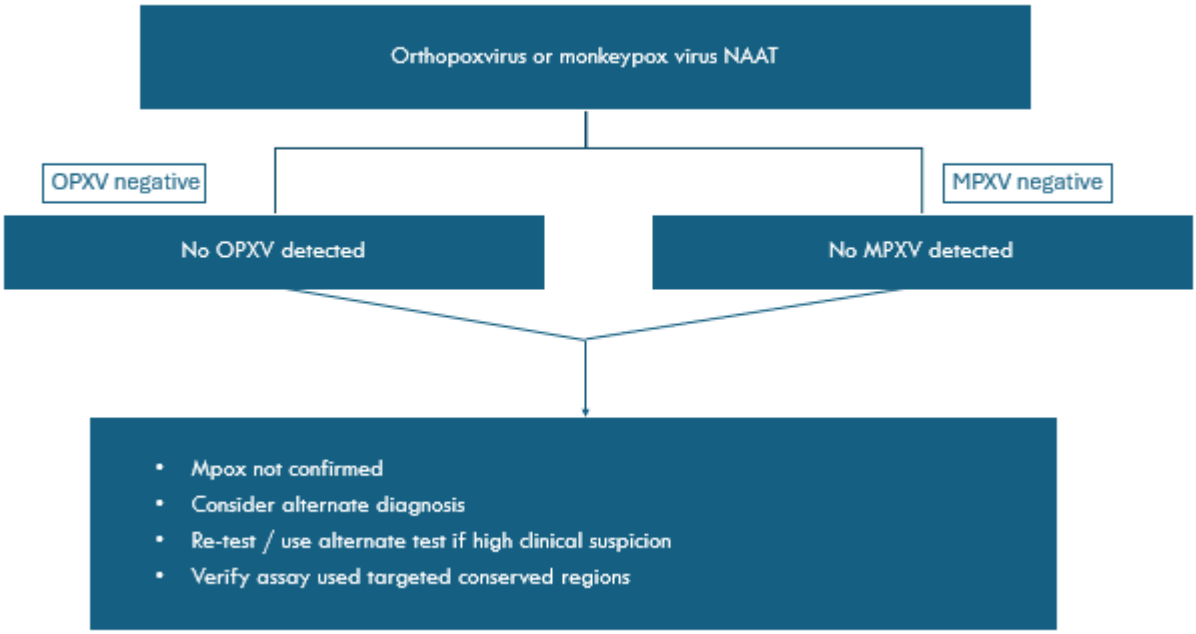

Figure 1: Laboratory testing algorithm for clinical management and surveillance of mpox: negative results.

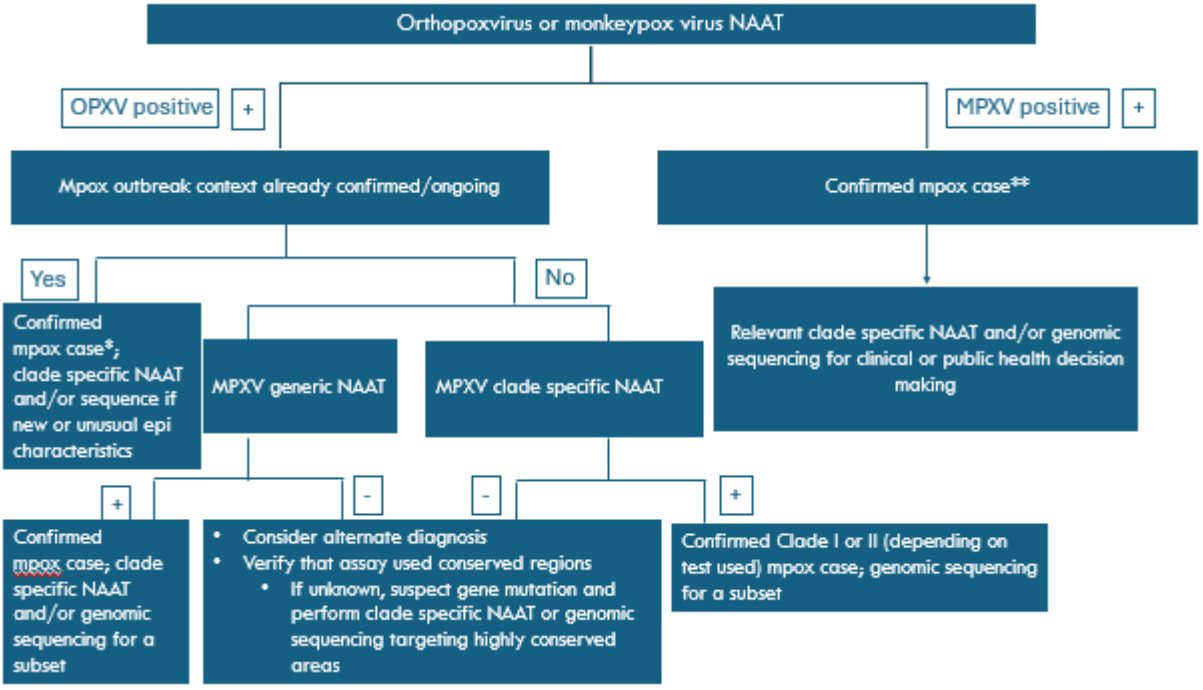

Figure 2: Laboratory testing algorithm for clinical management and surveillance of mpox: positive results.

*This applies in resource-limited settings and provided that other orthopoxviruses do not co-circulate in humans; otherwise, a MPXV-specific or MPXV clade-specific test is required for confirmation.

**if resources allow, sample should be further characterized in a reference laboratory

Several groups have validated PCR protocols for the detection of OPXV and more specifically MPXV, some of which include distinction of clades I (Congo Basin) and II (West African) viruses.(48–52) 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.(48–50) Some protocols involve two steps. In 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 use sequencing to confirm MPXV, specifically detect MPXV clades and, in the case of sequencing, lineages. Over the past year, several commercial PCR test kits detecting OPXV or specifically MPXV have become available, and performance evaluation studies have provided evidence on which of them have high sensitivity and specificity.(53,54) Positive control material for PCR assays can be ordered from specialized initiatives (55). For best practice, the positive control should be included at a low concentration which is unequivocally above the limit of detection. Inclusion of quality control materials where possible can assist in controlling for any assay issues. Controls should provide information about (1) specimen quality, (2) nucleic acid quality, and (3) process quality. Because PCR can be extremely sensitive, efforts should be made to avoid contamination, and negative controls should be used on every run to ensure contamination has not occurred. Specimen integrity controls (e.g. RNase P), and extraction, positive and inhibition controls can help in distinguishing a false negative from a true negative. Controls should be used following laboratory SOPs. If any of the assay controls fail, testing should be repeated. Criteria set in the target product profiles for tests to be used in mpox diagnosis can be reviewed to support national procurement strategies.(2)

Electron microscopy. Electron microscopy can be used to evaluate the specimen for a potential orthopoxvirus. Considering the availability of molecular assays and the high technical skills and facility required for this method, it is not routinely used for the diagnosis of poxviruses, and cannot reliably distinguish between orthopoxvirus species.

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. The specific details for these methods are not covered in this document because they are not recommended as part of routine diagnosis.

Serology. Antibody detection from plasma or serum should not be used alone for clinical diagnosis of mpox. MPXV-specific antibody based tests are expected to face challenges of cross reactivity with antibodies to other orthopoxviruses as well as those elicited by vaccination.(56,57) WHO therefore recommends that serology testing should be restricted to reference laboratories until further evidence is available. Where a validated serological test is available in a reference laboratory, IgM detection from recently acutely ill patients or IgG in paired serum specimens–collected at least 14-21 days apart, with the first being collected during the first week of illness, can aid diagnosis if NAAT testing yields inconclusive results.

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, according to approved laboratory and national procedures.

Point of care testing

DNA detection. Point of care (POC) testing for MPXV is based on detection of nucleic acids, antigens and/or antibodies. Two molecular POC tests have received emergency use authorization (EUA) from the United States Food and Drug Administration(FDA).(58) One detects DNA from MPXV (clade II only) and non-variola orthopoxvirus in human lesion swab specimens (59) and the other detects DNA from MPXV (clades I/II) in human lesion swab specimens.(60) Both have been validated by manufacturers using an FDA-cleared real-time PCR test as the reference standard. The clinical validation was done using patient samples in the United States of America (confirmed PCR-positive for MPXV clade IIb only). Results of those evaluations are included in the instructions for use,[1] and they are comparable to the laboratory-based PCR reference standard. However, sample sizes are small, and there are no data on clinical or analytical performance reported in the peer-reviewed or pre-print literature at this time. Independent clinical evaluation of POC molecular assays, including the FDA EUA products, is underway, and results will be soon published. WHO strongly encourages further research to determine the diagnostic accuracy and utility of such critical tools in settings where MPXV clades I and/or II circulate. If proven accurate and useful, these products may be used when and where laboratory-based diagnosis is prohibitive due to lack of timely access to testing and/or when confirmatory diagnosis would influence clinical and public health decision making. WHO will issue an update to this guidance as soon as more evidence becomes available on their diagnostic accuracy.

Antigen and/or antibody detection. Over the past year, antigen and/or antibody detection rapid diagnostic tests have been commercialized.(61)There are no relevant published studies in the peer-reviewed or pre-print literature or products registered under emergency use listing/authorization as sources of data related to the performance of these assays. It is thus not yet known if they have sufficient accuracy to play a role in clinical management or surveillance of mpox. A comparative evaluation including a small number of commercial antigen detection assays is underway and includes settings where only clade I is known to circulate. Research to understand whether and how well antigen tests can be used and with which specimens is also ongoing. MPXV-specific antibody-based tests are expected to show cross reactivity with respect to other orthopoxviruses, including after vaccination with vaccinia-based smallpox and mpox vaccines. Until further evidence is available, WHO does not recommend use of these tests for diagnosis of acute or past infection with MPVX.

Interpretation of testing results

Confirmation of MPXV infection should consider clinical and epidemiological information. Positive detection using an MPXV PCR assay, or using an initial OPXV PCR assay followed by confirmation of MPXV via PCR and/or sequencing, indicates confirmation of MPXV infection.

[1] 5-EUA230004 Cue Mpox (Monkeypox) Molecular Test IFU 03-17-2023_Cue (1) (cuehealth.com); Xpert Mpox (cepheid.com)

Positive detection using OPXV PCR assay alone is generally considered insufficient for laboratory confirmation of mpox, particularly in countries where there is co-circulation of other OPXVs. Currently, the WHO mpox case definition considers an OPXV-positive case as a probable case.

A number of factors could contribute to false-negative results, such as poor quality of specimen, inappropriate handling or shipping, or technical reasons inherent to the test, such as DNA extraction failure or operator error. In the case of persistently high clinical suspicion and lack of an alternative diagnosis, repeat testing should be considered. Gene deletions may also lead false negative results (12,18)

For epidemiological purposes, WHO will propose case definitions for reinfection (updated surveillance guidance, in preparation) and these should be taken into consideration in test result interpretation.

In August 2023, Standing recommendations for mpox were issued by the Director-General of WHO in accordance with the International Health Regulations (2005) (IHR). These Standing recommendations call on all States Parties to include mpox as a notifiable disease in the national epidemiological surveillance system; to strengthen diagnostic capacity at all levels of the health care system; to ensure timely reporting of cases to WHO, as per WHO guidance and case reporting form; and to share genetic sequence data and metadata through public databases.(62)

Genomic sequencing

In addition to the potential 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, the origins of cases which are not part of the clade IIb B.1 lineage that gave rise to the 2022-2023 and ongoing multi-country outbreak or new strains emerging in countries where mpox is known to be endemic. (63,64) GSD for MPXV does not provide the same resolution to track chains of transmission as it does for RNA viruses (such as Ebola virus). Nonetheless, a strategic and representative approach to sequencing of positive cases remains very valuable for understanding the epidemiology of mpox in a country. Targeted sequencing should be considered in specific situations, such as for imported cases, re-infections, mutations leading to diagnostic failure or suspected enhanced human to human transmission, or vaccine breakthrough cases, and to assess for antiviral resistance.(65)

WHO strongly encourages countries and laboratories to share GSD, including raw data whenever possible, in a timely manner through the available open access databases.

Testing for HIV

Persons living with HIV who are immunosuppressed are at higher risk of developing severe mpox disease.(66) Therefore, most particularly where cases and outbreaks may be linked to sexual transmission or any circumstance where immune suppression may be suspected or known to be present, patients with mpox for whom HIV status is not known should be tested for HIV per the current WHO consolidated guidance on HIV testing services.(29,67)

Biological risk management

It is recommended that all manipulations of specimens originating from suspected, probable or confirmed cases of mpox in the laboratory be conducted according to a risk-based approach. Each laboratory should conduct a local (institutional) risk assessment. When manipulating biological specimens, core biosafety requirements must be met (similar to those previously referred to as biosafety levels, see laboratory biosafety manual, 4th edition 2), and heightened control measures, indicatively equivalent to biosafety level 3, should be applied based on local risk assessment.(68)

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

- Specimens from persons who may have mpox must be handled in a functioning biosafety cabinet (Class I, II or III) prior to specimen inactivation. Properly inactivated specimens do not require a biosafety cabinet.
- Each laboratory should ensure that local inactivation protocols have been validated. The UK Health Security Agency (UKHSA) has undertaken assessments of inactivation methods against MPXV.(69)
- Laboratory personnel should wear appropriate personal protective equipment, especially for handling specimens before inactivation.(29)

- Where use of a centrifuge is required for a procedure on non-inactivated specimens, safety cups or sealed rotors should be used.

Additional control measures should be considered for specific procedures, including aerosol-generating 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. (68)

Occupational health and safety for health workers and laboratory personnel

There are currently three vaccines (LC16, MVA-BN, and OrthopoxVac) approved in different jurisdictions for prevention of mpox. These vaccines contain non-replicating (MVA-BN) or minimally-replicating (LC16, OrthopoxVac) strains of vaccinia virus, an orthopoxvirus long used as a vaccine to prevent smallpox (declared eradicated in 1980). The replication-competent vaccinia-based smallpox vaccine ACAM2000 or equivalent vaccines that meet WHO quality standards may also be considered.

All vaccinia virus-based vaccines provide cross-protection against other OPXV, including against mpox. (36,70)

For persons at risk of occupational exposure to orthopoxviruses, primary preventive (pre-exposure) vaccination (PPV) is recommended.(36) Therefore, national health authorities should conduct a risk assessment and consider immunization for individuals who may be at risk based on their clinical susceptibility and exposure risk and the availability of vaccine. This group may include health workers, laboratory personnel, persons working with wild animals in the field or in veterinary laboratories and other persons who may be at risk of exposure to MPXV.

Reporting of cases and test results

Laboratories should follow national reporting requirements and be particularly attentive regarding confirmed cases with a relevant recent history of international travel.(62). All MPXV test results, positive or negative, should be immediately reported to national authorities and WHO as per the standard case reporting form. The standing recommendations for mpox issued by the WHO Director-General in accordance with the International Health Regulations (2005) (IHR) outline current WHO recommendations for Member States.(62,71) 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).(72)

Global laboratory networking

Access to timely and accurate laboratory testing of specimens from cases under investigation is an essential part of the diagnosis and surveillance of this emerging infection. Countries should strengthen diagnostic capacity at all levels of the health system and at a minimum, should have access to reliable testing at national level or through referral to laboratories in other countries that are willing and able to perform OPXV or MPXV diagnostics. WHO, through its Regional Offices, can assist Member States in accessing testing through referral. Where appropriate and safely performed, inactivation of specimens in the local laboratory may facilitate referral and ease logistical challenges. The United States 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 methods for guidance development

This document was prepared by WHO in consultation with subject matter laboratory experts with experience handling and detecting MPXV and OPXV and individuals with expertise in public health virology and the development of diagnostic assays for OPXVs.

This emergency interim guidance was developed according to the standards and methods described in the *WHO eManual,* XVII.2.10 Clearance of Interim Guidance During Graded Emergencies. In June 2023, the WHO Secretariat convened an expert panel to review proposed updated recommendations to the interim guidance previously published on 23 May 2022. For this latest update an expert panel meeting was held on 12 March 2024.

For countries that have regulatory standards that apply to clinical laboratory testing performed on human specimens, those regulatory standards should appropriately be followed.

Stepwise approach

Step 1: Define key questions needing update. The WHO Secretariat held preparatory conference calls with five key expert groups from various countries to identity and list key questions for which there was a need to review latest evidence. The Secretariat also reviewed and revised interim guidance documents for the mpox outbreak response to ensure relevant updates are noted and aligned.

Step 2: Review evidence. A comprehensive search using one search string for each question was performed online via PubMed. Because of the accelerated timeline and broad scope of the guideline, it was not feasible to undertake a formal GRADE process (PICO questions; systematic reviews; formal documentation of values and preferences and incorporation of considerations of costs, resources, and feasibility).

Step 3. Convene expert group meeting. On 28 June 2023, WHO convened an expert group comprised of a multidisciplinary panel of virologists, scientists, public health officials, and clinicians with experience in the diagnosis of patients with emerging zoonotic diseases including orthopoxviruses. In preparation for this meeting, the previous interim guidance was annotated and circulated to the panel.

Step 4: Prepare updated recommendations. The expert group was convened and was moderated by the WHO Laboratory Lead for the global mpox response. Draft recommendations were shared with the panel in advance and discussion was moderated until consensus was achieved. If there was no clear consensus, this was captured in the draft document. The draft document was shared with the expert group in an iterative process for review. Information on the instructions for use of the two point of care tests that have received FDA emergency use authorization (EUA) approval was reviewed by WHO technical staff. Under confidential cover, WHO also reviewed manufacturers' submissions for FDA emergency use authorization (EUA) approval for both tests with FDA EUA.

Step 5: Review updated version. WHO updated the interim guidance to incorporate feedback from experts during the meeting and circulated the updated version with the expert group as well as to all responsible officers for each pillar of the WHO mpox response as well as with Regional Office laboratory focal points for mpox.

Step 6: Publish and disseminate. The final document was submitted and approved for WHO executive clearance.

Plans for updating

This interim guidance incorporates the latest understanding and characteristics of the monkeypox virus and addresses questions and issues received from WHO Country and Regional offices and other channels. WHO continues to monitor the situation closely for any changes that may affect this document. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance document will expire two years after the date of publication.

Contributors

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Declaration of interests

Experts in the network completed a confidentiality agreement and declaration of interest (DOI). During the meeting in November 2023, the WHO Secretariat described the DOI process, the outcome of its review and provided an opportunity to experts to declare any interests not provided in written form. No conflicts were declared. Web searches did not identify any additional interests that could be perceived to affect an individual's objectivity and independence during the development of the recommendations. 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 for diagnostic testing for mpox

Specimen type	Population	Collection materials	Storage temperature	Reference
For diagnosis				
Swab of lesion[2] material, including: – surface – exudate – crusts	All	Dacron or polyester flocked swabs with VTM or dry swab(73)	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	Gold standard for diagnosis: Lourie et al 1972, (74) CDC 1997.(75)
Oropharyngeal swab	All	Dacron or polyester flocked swabs with VTM or dry swab	See above	Tarin-Vicente et al 2022;(38) Suner et al 2023,(39) Palich et al 2023;(40) Ouafi et al 2023,(41) Edman-Waller et al 2023,(42) Paran et al 2023.(76)
Anorectal swab	Depending on clinical symptomatology and contact exposure	Dacron or polyester flocked swabs with VTM or dry swab	See above	Tarin-Vicente et al 2022;(38) Suner et al 2023,(39) Palich et al 2023;(40) Edman-Waller et al 2023.(42)

[2] Skin rash (papules, pustules, vesicles, crusts) or mucosa

Specimen type	Population	Collection materials	Storage temperature	Reference
Aid in diagnosis or for research purposes (following national ethics guidelines)				
Whole blood	All	Sterile collection tube with EDTA	See above	Suner et al 2023,(39) Palich et al 2023;(40) Edman-Waller et al 2023.(42)
Serum	All	Serum-separating tubes	See above	Karem et al 2005,(77) Hammarlund et al 2005,(78) Taub et al 2008,(79) Otter et al 2023.(57)
Plasma	All	Collection tube with EDTA	See above	Karem et al 2005, (77) Hammarlund et al 2005, (78) Taub et al 2008, (79) Otter et al 2023.(57)
For research purposes (following national ethics guidelines)				
Urine	All	Sterile collection tube	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	Palich et al 2023.(39)
Semen	Men	Sterile collection tube	Room temperature for <1h (then -20°C or lower)	Suner et al 2023,(39) Palich et al 2023.(40)
Vitreous fluid	Depending on clinical symptomatology and contact exposure	Sterile collection tube	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	Thornhill et al 2022.(26)
Cerebrospinal fluid	Depending on clinical symptomatology	Sterile collection tube	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	Badenoch et al 2022.(43)

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