Diagnostic testing for the monkeypox virus (MPXV)

Interim guidance

9 November 2023

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.
- 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.
- The presence of virus is confirmed by nucleic acid amplification testing (NAAT), such as real-time or conventional polymerase chain reaction (PCR).
- MPXV-specific and MPXV-clade specific NAAT 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.
- All handling in laboratory settings of specimens originating from suspected, probable or confirmed cases of mpox should be conducted under relevant biosafety conditions based on a risk-based approach.
- 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 or confirmed mpox.
- This is an updated version of the interim guidance on *Laboratory testing for the monkeypox virus* and supersedes the one published on 23 May 2022.

Changes from earlier version

This is an updated version of the interim guidance on *Laboratory testing for the monkeypox virus* and supersedes the one published on 23 May 2022. This version includes updated recommendations on alternative specimen types for testing, considerations for reducing risks of infection during specimen collection, and updated considerations around monkeypox virus (MPXV) versus orthopoxvirus (OPXV) laboratory confirmation. This version is in alignment with other updated interim guidance documents published by WHO since May 2022.

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 on the basis of its initial detection in 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 is further classified in clade IIa and clade IIb.(6) Clade II is the predominant strain in the current global outbreak, which has disproportionally affected men who have sex with men.

Mpox can cause a range of signs and symptoms. The incubation period of mpox has historically ranged from 5 to 21 days.(7) The classic mpox presentation is a short febrile prodromal phase, which lasts 1-5 days and during which time patients may experience fever, headache, back pain, muscle aches, and lymphadenopathy – which is a distinctive feature of this disease.(8) This is followed by a second phase that typically occurs after the fever subsides, with the appearance of skin and/or mucosal rash, which might include a single or multiple lesions.(9,10) Typically, the lesions progress through macules, papules, vesicles, and pustules, before crusting over and desquamating over a period of 2 to 4 weeks.(3)

In the context of the multi-country mpox outbreak (2022-2023), the recorded incubation period has ranged from as little as 1-3 days (11) to 10-12 days,(12) or occasionally much longer (as much as 40 days).(13) Patients have presented with more mucosal lesions than previously described, and often these are localized in the genital or perineal/perianal area as well as in the mouth and on the eyes.(14,15) An observational study reported that in half of patients, skin lesions were the first sign of infection.(16) Anorectal pain and bleeding (e.g. due to proctitis) has also been newly reported in this outbreak.(1) Lymphadenopathy remains a common feature, usually appearing early in the course of illness.(14)

Persons who have suspected, probable or confirmed mpox, as well as health workers and laboratory personnel, should take appropriate specific infection prevention and control (IPC) measures to prevent further spread. Detailed information and recommendations for clinical management, including treatment options and IPC, strategies can be found here.(17) 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 tests 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 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), herpes simplex virus (HSV), *Treponema pallidum* (syphilis), measles, vaccinia, parapoxviruses (causing orf and related conditions), molluscum contagiosum, disseminated gonococcal infection (DGI), scabies, vasculitis, bacterial skin and soft tissue infections, medication allergies and chancroid.(1,17,18)

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 may not guarantee full protection from future infection. Therefore, if an individual presents with clinical symptoms suggestive of mpox, it is crucial for them to promptly seek medical attention; undergo testing for mpox, HIV and other STIs as indicated; and receive appropriate medical care.(19) If resources are limited, high-risk groups such as children, pregnant women and 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.(20,21)

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 up to half of persons with confirmed mpox may also have previously undetected HIV infection, which heightens the importance of universal precautions.(22,23)

The WHO Strategic Advisory Group of Experts on immunization (SAGE) recommends primary preventive vaccination (PPV) 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).(24)

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.(17)

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.(25–29) 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.(14,26,27,30) 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 and any other applicable regulations depending on the mode of transport being used. For international transport, specimens from suspected, probable or confirmed mpox cases–including clinical specimens, viral isolates and cultures–should be transported as Category A, UN2814 “infectious substance, affecting humans”. Some countries have chosen to recategorize MPXV to UN3373 Category B via the Multilateral Agreement M347 under section 1.5.1 of ADR on the carriage of MPXV for road transport.

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.(31)

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, (32) 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), for detection of unique sequences of viral DNA. PCR can be used alone or in combination with sequencing for clade determination. Several groups have developed validated PCR protocols[1] for the detection of OPXV and more specifically MPXV, some of which include distinction of clades I (Congo Basin) and II (West African) viruses.(33–37) 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.(33–35) 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 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.(38,39) Positive control material for PCR assays can be ordered from specialized initiatives (40). For best practice, 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) 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 poxvirus. 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.

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 recent or even historical vaccination.(41,42) Therefore, WHO recommends that serology testing should be restricted to reference laboratories until further evidence is available on serological and/or antibody-detecting point of care (POC) tests for use outside such laboratories. In case 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 21 days apart, with the first being collected during the first week of illness–can aid diagnosis if tested specimens otherwise yield 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 specific laboratory 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).(43) One detects DNA from MPXV (clade II only) and non-variola orthopoxvirus in human lesion swab specimens(44) and the other detects DNA from MPXV (clades I/II) in human lesion swab specimens.(45) 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,[2] 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,

[1] 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

[2] 5-EUA230004 Cue Mpox (Monkeypox) Molecular Test IFU 03-17-2023_Cue (1) (cuehealth.com); Xpert Mpox (cepheid.com)

and results are expected in Q1 2024. 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.(46)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. Results are expected in early 2024. 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 OPXV PCR assay followed by confirmation of MPXV via PCR and/or sequencing or detection using MPXV PCR assay indicates confirmation of MPXV infection.

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 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.

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.

Standing recommendations for mpox, in accordance with the International Health Regulations (2005) (IHR), call on 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.(47)

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 whether cases are not part of the clade IIb B.1 lineage cluster that gave rise to the 2022-2023 multi-country outbreak. GSD 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, vaccine breakthrough cases, and prolonged infection in people under treatment, to assess for antiviral resistance.(48)

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

Testing for HIV

Persons living with HIV who are immunosuppressed are at higher risk of developing severe mpox disease.(49) Therefore, 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.(17,50)

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.(51)

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.(52)
- Laboratory personnel should wear appropriate personal protective equipment, especially for handling specimens before inactivation.(17)
- 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. (51)

Occupational health and safety for health workers and laboratory personnel

There are currently three vaccines (LC-16, MVA-BN, and OrthopoxVac) approved in different jurisdictions for prevention of mpox. These vaccines contain non-replicating (MVA-BN) or minimally-replicating 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 may also be considered.

All vaccinia virus-based vaccines provide cross-protection against other OPXV, including against mpox. (24,53)

For persons at risk of occupational exposure to orthopoxviruses, primary preventive (pre-exposure) vaccination (PPV) is recommended.(24) 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.(47). 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), outlines current WHO recommendations for Member States.(47,54) 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).(55)

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 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 beforehand 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

External contributors:

Daniel Bailey, Claire Gordon, and Ashley Otter, UKHSA, UK; Janine Michel and Andreas Nitsche, Robert Koch Institute, Germany; Chantal Reusken and Harry Vennema RIVM, Netherlands (Kingdom of the); Fabrizio Maggi and Giulia Matusali, Spallanzani Institute, Italy; Jacqueline Weyer, National Institute for Communicable Diseases, South Africa; Lateefat Amao, Nigeria Centre for Disease Control, Nigeria; Richard Njouom, Centre Pasteur du Cameroun, Cameroon; Jean Bernard Lekana-Douki and Nadine N'dilimabaka, Centre international des recherches médicales de Franceville, Gabon; Christina Hutson, US Centers for Disease Prevention and Control, Unites States of America; Christophe Batejat, Institut Pasteur, France; Supaporn Wacharapluesadee and Opass Putcharoen, Chulalongkorn University, Thailand; Isabella Anna Eckerle, Hôpitaux Universitaires de Genève, Switzerland; Francis Amirtharaj Selvaraj, Sheikh Khalifa Medical City, United Arab Emirates; Clarissa Damaso, Federal University of Rio de Janeiro, Brazil; Daniel Bausch, Foundation for Innovative New Diagnostics, Switzerland; Aytenew Eshete, Africa Centres for Disease Control and Prevention; Judith Wong, Raymond Lin and Lee Ching Ng, Ministry of Health, Singapore; Placide Mbala, Institut national de recherche biomédicale, Democratic Republic of the Congo; Pragya Yadav, National Institute of Virology of the Indian Council of Medical Research, India; Jen Kok, New South Wales Health Pathology - Institute of Clinical Pathology and Medical Research, Australia; Julian Druce, Deborah Williamson and Chuam Kok Lim, Victoria Infectious Disease Reference Laboratory, Australia.

WHO Headquarters and Regional Office staff contributors and technical review group: Lisa Carter, Jane Cunningham, Ana Hoxha, Kazunobu Kojima, Krutika Kuppalli, Rosamund Lewis, Jairo Mendez Rico, Bernadetta Basuta Mirembe, Karen Nahapetyan, Dhamari Naidoo, Bernard Onoja, James Richard Otieno, Jamie Rylance, Ute Stoeher, Lorenzo Subissi, Lara Vojnov, Karin Von Eije, Adesola Yinka-Ogunleye.

External contributors to the first interim guidance published on 23 May 2022: Lateefat Amao, Nigeria Centre for Disease Control, Nigeria; Daniel Bailey, UK Health Security Agency, United Kingdom of Great Britain and Northern Ireland; Meera Chand, UK Health Security Agency, United Kingdom; Francesca Colavita, Spallanzani Institute, Italy; Anna Rosa Garbuglia, Spallanzani Institute, Italy; Claire Gordon, UK Health Security Agency, United Kingdom; Susan Hopkins, UK Health Security Agency, United Kingdom; Marion Koopmans, Erasmus Medical Centre, Netherlands (Kingdom of the); Rinat Maksyutov, VECTOR, Russian Federation; Emmanuel Nakouné, Institut Pasteur Bangui, Central African Republic; Andreas Nitsche, Robert Koch Institute, Germany; Richard Njouom, Centre Pasteur du Cameroun, Cameroon; Tommy Rampling, UK Health Security Agency, United Kingdom; Chantal Reusken, RIVM, Netherlands (Kingdom of the); Jacqueline Weyer, National Institute for Communicable Diseases, South Africa.

Declaration of interests

Experts in the network completed a confidentiality agreement and declaration of interest (DOI). During the meeting, 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.

References

1. World Health Organzation. Surveillance, case investigation and contact tracing for mpox (monkeypox). 2022 Dec 22; Available from: Surveillance, case investigation and contact tracing for mpox (monkeypox)

2. World Health Organization. Target product profiles for tests used for mpox (monkeypox) diagnosis. 2023 Jul; Available from: https://apps.who.int/iris/handle/10665/371297

3. Jezek Z, Grab B, Szczeniowski MV, Paluku KM, Mutombo M. Human monkeypox: secondary attack rates. Bull World Health Organ. 1988;66(4):465–70.

4. Brown K, Leggat P. Human Monkeypox: Current State of Knowledge and Implications for the Future. TropicalMed. 2016 Dec 20;1(1):8.

5. Magnus P von, Andersen EK, Petersen KB, Birch-Andersen A. A POX-LIKE DISEASE IN CYNOMOLGUS MONKEYS. Acta Pathologica Microbiologica Scandinavica. 1959;46(2):156–76.

6. Ulaeto D, Agafonov A, Burchfield J, Carter L, Happi C, Jakob R, et al. New nomenclature for mpox (monkeypox) and monkeypox virus clades. The Lancet Infectious Diseases. 2023 Mar;23(3):273–5.

7. Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye A, Ihekweazu C, et al. Human Monkeypox: Epidemiologic and Clinical Characteristics, Diagnosis, and Prevention. Infect Dis Clin North Am. 2019 Dec;33(4):1027–43.

8. Pittman PR, Martin JW, Kingebeni PM, Tamfum JJM, Wan Q, Reynolds MG, et al. Clinical characterization of human monkeypox infections in the Democratic Republic of the Congo [Internet]. Infectious Diseases (except HIV/AIDS); 2022 May [cited 2023 Sep 5]. Available from: http://medrxiv.org/lookup/doi/10.1101/2022.05.26.22273379

9. Damon IK. Status of human monkeypox: clinical disease, epidemiology and research. Vaccine. 2011 Dec 30;29 Suppl 4:D54-59.

10. Adler H, Gould S, Hine P, Snell LB, Wong W, Houlihan CF, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. Lancet Infect Dis. 2022 Aug;22(8):1153–62.

11. Koch-Institut R. Kurze Inkubationszeiten bei Affenpockenfällen in Deutschland während des aktuellen Ausbruchsgeschehens. 2022 [cited 2023 Sep 5]; Available from: https://edoc.rki.de/handle/176904/10215

12. UK Health Security Agency. Mpox: transmission, infectious and incubation periods, and isolation guidance. 2022; Available from: https://www.gov.uk/government/publications/mpox-transmission-and-infectious-and-incubation-periods

13. Angelo KM, Smith T, Camprubí-Ferrer D, Balerdi-Sarasola L, Díaz Menéndez M, Servera-Negre G, et al. Epidemiological and clinical characteristics of patients with monkeypox in the GeoSentinel Network: a cross-sectional study. Lancet Infect Dis. 2023 Feb;23(2):196–206.

14. Thornhill JP, Barkati S, Walmsley S, Rockstroh J, Antinori A, Harrison LB, et al. Monkeypox Virus Infection in Humans across 16 Countries - April-June 2022. N Engl J Med. 2022 Aug 25;387(8):679–91.

15. Nguyen MT, Mentreddy A, Schallhorn J, Chan M, Aung S, Doernberg SB, et al. Isolated Ocular Mpox without Skin Lesions, United States. Emerg Infect Dis [Internet]. 2023 Jun [cited 2023 Sep 5];29(6). Available from: https://wwwnc.cdc.gov/eid/article/29/6/23-0032_article

16. Prasad S, Galvan Casas C, Strahan AG, Fuller LC, Peebles K, Carugno A, et al. A dermatologic assessment of 101 mpox (monkeypox) cases from 13 countries during the 2022 outbreak: Skin lesion morphology, clinical course, and scarring. Journal of the American Academy of Dermatology. 2023 May;88(5):1066–73.

17. World Health Organzation. Clinical management and infection prevention and control for monkeypox: Interim rapid response guidance. 2022 Jun 10; Available from: https://www.who.int/publications/i/item/WHO-MPX-Clinical-and-IPC-2022.1

18. World Health Organization. Managing epidemics: Key facts aboiut major deadly diseases. [Internet]. Available from: https://apps.who.int/iris/handle/10665/272442

19. World Health Organization. Multi-country outbreak of mpox, External situation report #23. Available from: https://www.who.int/publications/m/item/multi-country-outbreak-of-mpox--external-situation-report--23---26-may-2023

20. Van Dijck C, De Baetselier I, Kenyon C, Liesenborghs L, ITM Monkeypox Study Group, Vercauteren K, et al. Mpox screening in high-risk populations finds no asymptomatic cases. Lancet Microbe. 2023 Mar;4(3):e132–3.

21. Ferré VM, Bachelard A, Zaidi M, Armand-Lefevre L, Descamps D, Charpentier C, et al. Detection of Monkeypox Virus in Anorectal Swabs From Asymptomatic Men Who Have Sex With Men in a Sexually Transmitted Infection Screening Program in Paris, France. Ann Intern Med. 2022 Oct;175(10):1491–2.

22. Laurenson-Schafer H, Sklenovská N, Hoxha A, Kerr SM, Ndumbi P, Fitzner J, et al. Description of the first global outbreak of mpox: an analysis of global surveillance data. Lancet Glob Health. 2023 Jul;11(7):e1012–23.

23. World Health Organization. 2022-23 Mpox (Monkeypox) Outbreak: Global Trends. 2023 Jul 18; Available from: https://worldhealthorg.shinyapps.io/mpx_global/#5_Genomic_epidemiology

24. World Health Organization. Vaccines and immunization for monkeypox: Interim guidance, 16 November 2022. 2022 Nov 16; Available from: https://www.who.int/publications/i/item/WHO-MPX-Immunization

25. Tarín-Vicente EJ, Alemany A, Agud-Dios M, Ubals M, Suñer C, Antón A, et al. Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study. Lancet. 2022 Aug 27;400(10353):661–9.

26. Suñer C, Ubals M, Tarín-Vicente EJ, Mendoza A, Alemany A, Hernández-Rodríguez Á, et al. Viral dynamics in patients with monkeypox infection: a prospective cohort study in Spain. Lancet Infect Dis. 2023 Apr;23(4):445–53.

27. Palich R, Burrel S, Monsel G, Nouchi A, Bleibtreu A, Seang S, et al. Viral loads in clinical samples of men with monkeypox virus infection: a French case series. The Lancet Infectious Diseases. 2023 Jan;23(1):74–80.

28. Ouafi M, Regueme A, Alcaraz I, Riviere P, Bazus H, Salmon-Rousseau A, et al. Oropharyngeal samples versus lesion specimens at diagnosis in patients infected with monkeypox virus in Northern France. Journal of Medical Virology [Internet]. 2023 Jan [cited 2023 Jul 6];95(1). Available from: https://onlinelibrary.wiley.com/doi/10.1002/jmv.28276

29. Edman-Wallér J, Jonsson O, Backlund G, Muradrasoli S, Sondén K. Results of PCR Analysis of Mpox Clinical Samples, Sweden, 2022. Emerg Infect Dis. 2023 Jun;29(6):1220–2.

30. Badenoch JB, Conti I, Rengasamy ER, Watson CJ, Butler M, Hussain Z, et al. Neurological and psychiatric presentations associated with human monkeypox virus infection: A systematic review and meta-analysis. EClinicalMedicine. 2022 Oct;52:101644.

31. World Health Organization. WHO Guidance on regulations for the transport of infectious substances 2021-2022 [Internet]. Available from: https://www.who.int/publications/i/item/9789240019720

32. McCollum AM, Damon IK. Human Monkeypox. Clinical Infectious Diseases. 2014 Jan 15;58(2):260–7.

33. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. Journal of Virological Methods. 2010 Oct;169(1):223–7.

34. Schroeder K, Nitsche A. Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses. Molecular and Cellular Probes. 2010 Apr;24(2):110–3.

35. Maksyutov RA, Gavrilova EV, Shchelkunov SN. Species-specific differentiation of variola, monkeypox, and varicella-zoster viruses by multiplex real-time PCR assay. Journal of Virological Methods. 2016 Oct;236:215–20.

36. Ropp SL, Jin Q, Knight JC, Massung RF, Esposito JJ. PCR strategy for identification and differentiation of small pox and other orthopoxviruses. J Clin Microbiol. 1995 Aug;33(8):2069–76.

37. Espy MJ, Cockerill III FR, Meyer RF, Bowen MD, Poland GA, Hadfield TL, et al. Detection of smallpox virus DNA by LightCycler PCR. J Clin Microbiol. 2002 Jun;40(6):1985–8.

38. Michel J, Targosz A, Rinner T, Bourquain D, Brinkmann A, Sacks JA, et al. Evaluation of 11 commercially available PCR kits for the detection of monkeypox virus DNA, Berlin, July to September 2022. Eurosurveillance [Internet]. 2022 Nov 10 [cited 2023 Jul 6];27(45). Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2022.27.45.2200816

39. Papadakis G, Tran T, Druce J, Lim CK, Williamson DA, Jackson K. Evaluation of 16 molecular assays for the detection of orthopox and mpox viruses. Journal of Clinical Virology. 2023 Apr;161:105424.

40. European Virus Archive. Monkeypox virus [Internet]. Available from: https://www.european-virus-archive.com/search/node/monkeypox

41. Edghill-Smith Y, Golding H, Manischewitz J, King LR, Scott D, Bray M, et al. Smallpox vaccine–induced antibodies are necessary and sufficient for protection against monkeypox virus. Nat Med. 2005 Jul 1;11(7):740–7.

42. Otter AD, Jones S, Hicks B, Bailey D, Callaby H, Houlihan C, et al. Monkeypox virus-infected individuals mount comparable humoral immune responses as Smallpox-vaccinated individuals. Nat Commun. 2023 Sep 23;14(1):5948.

43. US Food and Drug Administration. Monkeypox (mpox) Emergency Use Authorizations for Medical Devices. 2023 May 24; Available from: https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices

44. US Food and Drug Administration. Xpert Mpox - Letter of Authorization. 2023 Feb 10; Available from: https://www.fda.gov/media/165317/download

45. US Food and Drug Administration. Cue Mpox (Monkeypox) Molecular Test - Letter of Authorization. 2023 Mar 17; Available from: https://www.fda.gov/media/166359/download

46. Foundation for Innovative New Diagnostics. Monkeypox test directory. 2023 Jul 23; Available from: https://www.finddx.org/tools-and-resources/dxconnect/test-directories/monkeypox-test-directory/

47. World Health Organization. Standing recommendations for mpox issued by the Director-General of the World Health Organization (WHO) in accordance with the International Health Regulations (2005) (IHR). 2023 Aug 21; Available from: https://www.who.int/publications/m/item/standing-recommendations-for-mpox-issued-by-the-director-general-of-the-world-health-organization-(who)-in-accordance-with-the-international-health-regulations-(2005)-(ihr)

48. Smith TG, Gigante CM, Wynn NT, Matheny A, Davidson W, Yang Y, et al. Resistance to anti-orthopoxviral drug tecovirimat (TPOXX®) during the 2022 mpox outbreak in the US [Internet]. Public and Global Health; 2023 May [cited 2023 Jul 17]. Available from: http://medrxiv.org/lookup/doi/10.1101/2023.05.16.23289856

49. Mitjà O, Alemany A, Marks M, Lezama Mora JI, Rodríguez-Aldama JC, Torres Silva MS, et al. Mpox in people with advanced HIV infection: a global case series. The Lancet. 2023 Mar;401(10380):939–49.

50. World Health Organization. Consolidated guidelines on HIV testing services. 2019; Available from: https://apps.who.int/iris/handle/10665/336323

51. World Health Organization. Laboratory Biosafety Manual, 4th edition [Internet]. 2020. Available from: https://www.who.int/publications/i/item/9789240011311

52. UK Health Security Agency. Monkeypox: UKHSA laboratory assessments of inactivation methods. 2022 Aug 22; Available from: https://www.gov.uk/government/publications/monkeypox-ukhsa-laboratory-assessments-of-inactivation-methods

53. Deputy NP, Deckert J, Chard AN, Sandberg N, Moulia DL, Barkley E, et al. Vaccine Effectiveness of JYNNEOS against Mpox Disease in the United States. N Engl J Med. 2023 Jun 29;388(26):2434–43.

54. World Health Organization. Fifth Meeting of the International Health Regulations (2005) (IHR) Emergency Committee on the Multi-Country Outbreak of mpox (monkeypox). 2023 May 11; Available from: https://www.who.int/news/item/11-05-2023-fifth-meeting-of-the-international-health-regulations-%282005%29-%28ihr%29-emergency-committee-on-the-multi-country-outbreak-of-monkeypox-%28mpox%29

55. World Health Organization. International Health Regulations (2005) Third Edition [Internet]. Available from: https://www.who.int/publications/i/item/9789241580496

56. Lieberman NAP, Mathias PC, Bradley BT, Greninger AL. Clinical Performance and Trends during the First Two Months of Monkeypox Virus PCR Testing at Two United States Reference Labs. J Clin Microbiol. 2022 Dec 21;60(12):e0137122.

57. Lourie B, Bingham PG, Evans HH, Foster SO, Nakano JH, Herrmann KL. Human infection with monkeypox virus: laboratory investigation of six cases in West Africa. Bull World Health Organ. 1972;46(5):633–9.

58. Centers for Disease Control and Prevention (CDC). Human monkeypox -- Kasai Oriental, Democratic Republic of Congo, February 1996-October 1997. MMWR Morb Mortal Wkly Rep. 1997 Dec 12;46(49):1168–71.

59. Paran N, Yahalom-Ronen Y, Shifman O, Lazar S, Ben-Ami R, Yakubovsky M, et al. Monkeypox DNA levels correlate with virus infectivity in clinical samples, Israel, 2022. Euro Surveill. 2022 Sep;27(35):2200636.

60. Karem KL, Reynolds M, Braden Z, Lou G, Bernard N, Patton J, et al. characterization of acute-phase humoral immunity to monkeypox: use of immunoglobulin M enzyme-linked immunosorbent assay for detection of monkeypox infection during the 2003 North American outbreak. Clin Diagn Lab Immunol. 2005 Jul;12(7):867–72.

61. Hammarlund E, Lewis MW, Carter SV, Amanna I, Hansen SG, Strelow LI, et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. Nat Med. 2005 Sep;11(9):1005–11.

62. Taub DD, Ershler WB, Janowski M, Artz A, Key ML, McKelvey J, et al. Immunity from smallpox vaccine persists for decades: a longitudinal study. Am J Med. 2008 Dec;121(12):1058–64.

Annex

Specimen collection and storage for diagnostic testing for mpox

Specimen type	Population	Collection materials	Storage temperature	Reference
For diagnosis				
Swab of lesion[3] material, including: – surface – exudate – crusts	All	Dacron or polyester flocked swabs with VTM or dry swab(56)	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, (57) CDC 1997.(58)
Oropharyngeal swab	All	Dacron or polyester flocked swabs with VTM or dry swab	See above	Tarin-Vicente et al 2022;(25) Suner et al 2023,(26) Palich et al 2023;(27) Ouafi et al 2023,(28) Edman-Waller et al 2023,(29) Paran et al 2023.(59)
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;(25) Suner et al 2023,(26) Palich et al 2023;(27) Edman-Waller et al 2023.(29)

[3] 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,(26) Palich et al 2023;(27) Edman-Waller et al 2023.(29)
Serum	All	Serum-separating tubes	See above	Karem et al 2005,(60) Hammarlund et al 2005,(61) Taub et al 2008,(62) Otter et al 2023.(42)
Plasma	All	Collection tube with EDTA	See above	Karem et al 2005, (60) Hammarlund et al 2005, (61) Taub et al 2008, (62) Otter et al 2023.(42)
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.(26)
Semen	Men	Sterile collection tube	Room temperature for <1h (then -20°C or lower)	Suner et al 2023,(26) Palich et al 2023.(27)
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.(14)
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.(30)

WHO reference number: WHO/MPX/Laboratory/2023.1