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Centres for Disease Control and Prevention

World Health Organization
African Region

Mpox Testing Strategy

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ABBREVIATIONS AND ACRONYMS

Africa CDC	Africa Centres for Disease Control and Prevention
AFLTWG	Africa Laboratory Technical Working Group
Ag-RDT	Antigen- Rapid Diagnostic Test
AIDs	Acquired Immunodeficiency Syndrome
AMA	Africa Medicines Agency
AU	African Union
BSL	Biosafety Level
BSC	Biosafety Cabinet
CFR	Case Fatality Rate
CDC	Centers for Disease Control and Prevention
DAC	Diagnostics Advisory Committee
DRC	Democratic Republic of the Congo
DNA	Deoxyribonucleic Acid
EQA	External Quality Assessment
EUL	Emergency Use List
GISAID	Global Initiative on Sharing All Influenza Data
GSD	Genetic Sequence Data
IHR	International Health Regulations
IVD	In Vitro Diagnostics
KPI	Key performance Indicator
M&E	Monitoring and Evaluation
MPOx	Previously known as monkeypox
MPXV	Monkeypox Virus
mPOC	molecular Point of Care
MVA-BN	Modified Vaccinia Ankara-Bavarian Nordic
NAAT	Nucleic Acid Amplification Tests
NGS	Next-Generation Sequencing
NIST	National Institute of Standards and Technology
NRL	National Reference Laboratory
NPHIs	National Public Health Institutes
OPxV	Orthopoxvirus
PHECS	Public Health Emergency of Continental Security
PHEIC	Public Health Emergency of International Concern
PPE	Personal Protective Equipment
PT	Proficiency testing
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SOP	Standard Operating Procedure
VTM	Viral Transport Medium
WHO	World Health Organization
WGS	Whole Genome Sequencing

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EXECUTIVE SUMMARY

Mpox testing and genome sequencing are critical for case identification, contact tracing, better characeriztion of strains and inform development of medical counter measures to control the epidemic. The rapidly changing epidemiological landscape of the mpox outbreak requires adaptable strategies and timely interventions to effectively respond to its spread and impact on public health. The mpox Testing Strategy therefore aims to enhance public health responses to mpox outbreak through strengthening of the laboratory pillar of the joint continental response plan. This strategy is critical in the context of rising mpox cases and its spread to more countries. There are several limitations in expanding diagnostic capacity in affected countries including very weak laboratory infrastructure especially at sub-national levels, challenges related to accessing quality assured diagnostics and absence of easily deployable point- of- care tests. Real time Polymerase Chain Reaction (RT-PCR) remains the main modality of diagnosis for mpox but this is only available in few centralized laboratories. Meeting the testing demand through centralized testing is very difficult because of the complexity of implementing a coordinated national specimen transportation especially in countries with difficult terrain and limited road networks. Near-Point-of-Care Nucleic Acid Ampilfication Tests (NAATs) such as GeneXpert testing networks are key to supplement centralized testing for expansion of services to sub-national levels.

During the initial emergency response to mpox, several key actions and strategies were implemented to address the outbreak effectively. A comprehensive joint continental response plan has been developed and its implementation is being co-led by Africa CDC and WHO. Under the laboratory pillar of the response plan, key early measures were taken including expanding access to diagnostic services through training and technical support, procurement and distribution of PCR test kits and GeneXeprt cartridges, support for specimen transportation and application of viral genome sequencing to monitor the distribution of clades.

The purpose of the testing strategy for mpox is to guide African Union (AU) Member States as they embark on strengthening their laboratory diagnostics and pathogen genome sequencing capabilities to effectively detect, manage, and control mpox outbreak. This strategy aims to provide a structured approach in planning for expansion of mpox testing and genome sequencing to respond to the outbreak quickly and effectively.

The guidance on testing strategy for mpox recommends to Africa Union Member States and partners several key actions in different areas of testing capacity expansion process to support optimal response to the current outbreak and to enhance capabilities to address future public health threats. The strategy describes the laboratory diagnostic methods and their limitations, practical considerations for introduction of mpox testing, integration of testing, sampling strategy and sample transportation, elaborates the laboratory testing algorithm in interpretation of test results, provides considerations for decentralization of mpox testing, describes sequencing and genomic surveillance strategies and quality of testing and safety considerations.

This testing strategy for mpox is a guide for stakeholders supporting the mpox response including Ministries of Health and other Government agencies, national mpox response task-forces, funding and implementing agencies, public health experts, epidemiologists, front-line healthcare service providers including clinicians, nurses, laboratory professionals, community healthcare workers and others.

The Africa CDC anticipates the successful adoption and implementation of this guidance to enhance the response to the mpox outbreak and strengthen public health efforts across Africa.

1. BACKGROUND AND RATIONALE

Mpox is an emerging zoonotic disease caused by the mpox virus, a member of the Orthopoxvirus genus closely related to the variola virus that causes smallpox. Mpox was first discovered in 1958 when outbreaks of a pox-like disease occurred in monkeys kept for research. The first human case was recorded in 1970 in the Democratic Republic of the Congo (DRC) during a period of intensified effort to eliminate smallpox and since then the infection has been reported in a number of African countries.[1&2] Mpox can spread in humans through close contact, usually skin-to-skin contact, including sexual contact, with an infected person or animal, as well as with materials contaminated with the virus such as clothing, beddings and towels, and respiratory droplets in prolonged face to face contact. People remain infectious from the onset of symptoms until all the lesions have scabbed and healed. The virus may spread from infected animals through handling infected meat or through bites or scratches. Diagnosis is confirmed by polymerase chain reaction (PCR) testing of material from a lesion for the virus's DNA.[3&4] Two separate clades of the mpox virus are currently circulating in Africa: Clade I, which includes subclades Ia and Ib, and Clade II, comprising subclades IIa and IIb. Clade Ia and Clade Ib have been associated with ongoing human-to-human transmission and are presently responsible for outbreaks in the Democratic Republic of the Congo (DRC), while Clade Ib is also contributing to outbreaks in Burundi and other countries.[5]

In 2022–2023 mpox caused a global outbreak in over 110 countries, most of which had no previous history of the disease, primarily driven by human-to-human transmission of clade II through sexual contact. In just over a year, over 90,000 cases and 150 deaths were reported to the WHO.[6] For the second time since 2022, mpox has been declared a global health emergency as the virus spreads rapidly across the African continent. On 13 Aug 2024, Africa CDC declared the ongoing mpox outbreak a Public Health Emergency of Continental Security (PHECS), marking the first such declaration by the agency since its inception in 2017.[7] This declaration empowered the Africa CDC to lead and coordinate responses to the mpox outbreak across affected African countries. On August 14, 2024, the WHO declared the resurgence of mpox a Public Health Emergency of International Concern (PHEIC) emphasizing the need for coordinated international response.[8]

As of August 2024, Mpox has expanded beyond its traditional endemic regions, with new cases reported in countries including Sweden, Thailand, the Philippines, and Pakistan. Sweden has confirmed its first case of Clade 1 variant, which has been rapidly spreading in Africa, particularly in DRC.[9] The emergence of this new variant raises concerns about its potential for higher lethality and transmission rates outside Africa.[10]

The rise in cases, especially in non-endemic regions, has prompted the need for effective surveillance and control measures to prevent further spread and manage cases promptly. African countries are implementing various public health measures, including contact tracing, enhanced surveillance, and community engagement strategies to mitigate further spread. To effectively manage mpox cases and prevent outbreaks, it is crucial to adopt a comprehensive strategy that includes improved testing, strong surveillance, clear communication, coordination, preventive actions, and continuous research. This well-rounded approach ensures that public health systems are ready to tackle the challenges presented by emerging infectious diseases such as mpox.

Developing an mpox testing strategy is integral to controlling the disease, protecting public health, and ensuring that appropriate measures are taken to manage and mitigate the impact of the virus.

[1]https://www.cdc.gov/poxvirus/mpox/about/index.html
[2]https://www.who.int/news-room/fact-sheets/detail/mpox
[3]https://africacdc.org/disease/monkeypox/
[4]https://www.who.int/news-room/questions-and-answers/item/mpox
[5]https://africacdc.org/download/africa-cdc-weekly-event-based-surveillance-report-august-2024/
[6]https://openwho.org/courses/mpox-global-outbreak-2023
[7]https://africacdc.org/news-item/africa-cdc-declares-mpox-a-public-health-emergency-of-continental-security-mobilizing-resources-across-the-continent/
[8]https://www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern
[9]https://www.ecdc.europa.eu/sites/default/files/documents/mpox-risk-assessment-monkeypox-virus-africa-august-2024.pdf
[10] https://africacdc.org/download/africa-cdc-weekly-event-based-surveillance-report-august-2024/

In essence, the testing strategy is designed to be used by a broad network of individuals and organizations involved in health care, public health, research and emergency response to effectively manage and control mpox.

Rationale

This mpox testing strategy is essential for the prompt detection and management of the disease, and serves to guide AU Member States in implementing effective testing protocols to facilitate the swift identification of cases. This is vital for the initiation of immediate public health measures, such as the isolation of infected individuals and the containment of spread. The strategy should prioritize testing individuals based on clinical symptoms and exposure risk, with a focus on those presenting with fever, rash and lymphadenopathy, especially if they have a history of contact with confirmed cases or travelled to endemic regions. Accurate diagnosis relies on highly sensitive PCR tests. Testing protocols must include clear case definitions, proper sample collection methods, and designated testing facilities equipped for safe and prompt analysis and return of results.

Practical considerations for the introduction of mpox testing is essential, incorporating testing for public health purposes to ensure healthcare providers and the public are informed about testing procedures and preventive measures and addressing limitations of testing. Sequencing and genomic surveillance, quality assurance for mpox testing and sequencing, data management, safety and ethical considerations, monitoring and evaluation and adaptation of the strategy are necessary to address emerging trends and improve overall response.

2. OBJECTIVES OF TESTING

- To confirm mpox in all symptomatic individuals that fulfil testing criteria through deployment of appropriate and accurate diagnostics technologies and for better characterization of mpox clades
- To support surveillance system to identify, isolate and manage cases and trace contacts and implement other public health measures to control mpox outbreak.
- Collect and analyze data on testing outcomes and trends to inform public health responses and refine testing strategies.

3. METHODS FOR MPOX TESTING AND LIMITATIONS

3.1 Laboratory Diagnostics of Mpox

Mpox laboratory testing should be ordered for any individuals meeting the case definitions for suspected or probable mpox disease. Laboratory testing for mpox virus (MPXV) should be performed in appropriately equipped laboratories by staff trained in relevant technical and safety procedures and conducted under relevant biosafety conditions using a risk-based approach.

3.2 Laboratory Diagnostic Methods

Testing method	Use cases
Laboratory-based NAAT	Gold-standard for the detection and confirmation of mpox cases. Can be used to detect and differentiate Orthopox viruses as well as mpox virus clades alone or in conjunction with genomic sequencing. Single or multi-step NAAT may be implemented depending on testing and public health goals, and can include clade-specific NAATs

Testing method	Use cases
Molecular point of care (mPOC)tests	When available, may be used to rapidly detect mpox cases to influence clinical care and public health interventions. Most appropriate in outbreaks or resource-limited settings where referral and testing of all suspected cases by the National Reference Laboratory (NRL) is not feasible. The near-point of care tests used for TB and HIV programs can give better options for rapid expansion of mpox testing in these settings
Mpox Rapid antigen tests	When available, can help to dencentralize testing to remote sites and support expansion of testing services for enhanced surveillance. There are a number of evaluations to determine performance of rapid antigen tests for mpox but none have been approved to be used for case detection so far.
Serology	May be used for operational research or retrospective studies. Not currently recommended for diagnosis or confirmation of mpox cases
Genomic Sequencing	In addition to the potential use of sequencing for diagnosis, genomic sequence data may also provide valuable information to help understand the origins, epidemiology, evolution, and characteristics of the virus and its different clades.

Nucleic acid amplification testing.

Africa CDC and WHO recommend confirmation of MPXV infection with laboratory-based 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 whether a single or multi-step PCR strategy is required, will depend on the local epidemiological context, in particular, whether differentiation of monkeypox virus clades is required for testing objectives, or whether other Orthopox viruses are suspected of co-circulating.

It is critical that any NAATs used are designed to detect highly conserved regions of the genome to reduce the risk of missing cases due to genomic deletions. For the same reason, it is also preferable to use NAATs which include multiple gene targets. Highly conserved NAATs can consist of a generic OPXV or MPXV PCR, which can be followed by MPXV specific or clade specific NAATs for positive samples for confirmation or epidemiological investigation respectively. If clade specific NAATs fail to detect mpox, subsequent sequencing can be used for clade determination and genetic characterization.[11] For a potential workflow see *Figures 1 and 2.*

Several groups have validated PCR protocols for the detection of OPXV and more specifically MPXV, some of which include distinction of clades I and II viruses or their subclades. 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. Some protocols involve two steps; first, PCR reaction detects OPXV but does not identify which species.[12] This can then be followed by a second step, which can be PCR-based or use sequencing to confirm MPXV, specifically to detect MPXV clades and, in the case of sequencing, lineages. 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. [13]

Positive control material for PCR assays can be ordered from specialized initiatives such as the WHO biohub or others. 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 can assist in controlling for any assay issues. Controls should provide information about specimen quality, nucleic acid quality, and 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

[11] mpox-dcp-v3.2.pdf (who.int)
[12] WHO-MPX-Laboratory-2023.1.eng.pdf
[13] Evaluation and clinical validation of monkeypox (mpox) virus real-time PCR assays - PMC (nih.gov)

extraction, positive and inhibition controls can help in distinguishing false negatives from true negatives. 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. [14]

Minimal and preferred targets for the performance of laboratory-based NAATs are outlined in the WHO Target Product Profile for Mpox . 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 [15].

The Africa CDC Diagnostic Advisory Committee (DAC) has undertaken a critical review of molecular tests for mpox and published two editions of list of recommended PCR test kits to guide Member States and partners in selection and procurement of test kits.

Molecular Point-of-Care Testing (DNA detection).

Molecular point of care (mPOC) testing for MPXV is based on detection of nucleic acids, but which can be performed in a decentralized setting by non-laboratory trained health care professionals and/or lay health workers near a patient, and outside the laboratory setting. Two mPOC tests have received emergency use authorization (EUA) from the United States Food and Drug Administration (FDA), of which only one is still being manufactured.[16] This test detects DNA from MPXV (clade II only) and non-variola orthopoxvirus in human lesion swab specimens and was 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 these evaluations are included in the instructions for use and are comparable to the laboratory-based PCR reference standard. Additional independent and manufacturer sponsored clinical evaluations of this, and other non-FDA approved mPOC, have also been completed (Annex 2, Figure 1).[17] Few mPOC devices showed an acceptable level of accuracy in its results and can be considered in testing decentralization strategies, however the limited number of datasets, heterogeneity in reference standards and test thresholds require careful roll out of such tools, including field validation in new contexts. Such issues make it difficult to make any more specific recommendations at this time. If mPOC are deployed, countries should have a system in place to ensure quality through proficiency testing, as well as capture results into diagnostic and surveillance pathways. More operational research is needed to determine the diagnostic accuracy and utility of such critical tools in settings where MPXV clades I and/or II circulate.

Antigen and/or antibody detection.

Rapid antigen tests for mpox have a very good potential to expand mpox testing to peripheral sites to strengthen surveillance in community settings. However; there are no good such technolgoies today for immediate deployment and hence additional research is needed to evaluate antigen tests on fresh samples. MPXV-specific antibody-based tests are expected to show cross reactivity with other orthopoxviruses, including after vaccination with vaccinia-based smallpox and mpox vaccines and hence the utility of such tests has not been well established except for some epidemiological studites that focus on determination of past exposure to mpox.

[11] mpox-dcp-v3.2.pdf (who.int)
[12]WHO-MPX-Laboratory-2023.1.eng.pdf
[13]Evaluation and clinical validation of monkeypox (mpox) virus real-time PCR assays - PMC (nih.gov)
[14]Xpert Mpox (cepheid.com)
[15] Masirika LM, Udahemuka JC, Schuele L, Ndishimye P, Otani S, Mbiribindi JB, et al. Ongoing mpox outbreak in Kamituga, South Kivu province, associated with monkeypox virus of a novel Clade I sub-lineage, Democratic Republic of the Congo, 2024. Eurosurveillance [Internet]. 2024 Mar 14 [cited 2024 Apr 29];29(11). Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.11.2400106
[16]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.
[17]FIND. Our evaluations of mpox diagnostics. Available from: https://www.finddx.org/what-we-do/programmes/pandemic-threats/mpox/diagnostics-evaluations/

4. PRACTICAL CONSIDERATIONS FOR THE INTRODUCTION OF MPOX TESTING

Introducing mpox testing requires a comprehensive approach that considers public health systems, regulatory frameworks, logistical challenges, and community engagement. Since the healthcare infrastructure varies significantly across countries and between regions, it is critical to tailor strategies to ensure that testing is accessible, effective and sustainable. It is also important to consider different response strategies for countries in high-burden and low-burden scenarios.

4.1 Regulatory and Compliance Requirements

Regulatory approval and compliance are fundamental. Countries must ensure testing kits and protocols are approved by relevant bodies such as national health ministries, WHO, and the African Medicines Agency (AMA). Africa CDC has also recently published a list of recommended molecular tests to support access to PCR tests. However, countries should establish clear guidelines for the validation and use of test kits. Harmonizing regulations across African countries can streamline processes, allowing easier procurement of test kits and resources across borders. Regulatory bodies should monitor and update standards as more is learned about the virus especially in the case of viral mutations that could affect diagnostic accuracy.

4.2 Laboratory Infrastructure

Given the varied capacities of laboratories across countries, it is critical to assess existing infrastructure before introducing mpox testing. The introduction of nucleic acid amplification testing (NAAT), such as real-time or conventional polymerase chain reaction (PCR), requires equipped laboratories with skilled personnel. Laboratories must have the capacity to handle molecular testing, including biosafety cabinets and other necessary materials to handle biohazardous samples safely and ensure the integrity of samples. Countries should take into account available capacity from investments made during the COVID-19 pandemic as well as the laboratory infrastructure built to support HIV, TB and other health conditions of public health priority. Concerted efforts should be made in increasing availability of multi-disease diagnostic platforms, point-of-care (POC) testing technologies and digital diagnostic tools that can enhance coverage.

In **high-burden regions**, where transmission rates are high or widespread outbreaks are occurring, rapid scaling up of laboratory capacity is essential. Therefore, integration of testing for outbreaks with TB and HIV molecular testing capacities can help fill this gap quickly. Mpox testing may be quickly integrated into these diagnostic facilities, as they are already equipped to manage similar molecular diagnostic processes. As clinical symptoms of mpox are similar to many diseases such as chickenpox and herpes, in high-burden countries, it is also important to integrate testing in existing algorithms to ensure people are getting diagnosed wherever they seek care. In low-burden regions, the focus should be on maintaining preparedness.

4.3 Test Accessibility

Ensuring equitable access to mpox testing is crucial. Decentralized testing models, mobile testing units and collaborations with local healthcare facilities can make testing accessible to underserved areas. Testing must be affordable or free for at-risk populations, especially in high burden settings.

Expanding laboratory coverage in both high- and low-burden areas is a priority for ensuring equitable access to mpox diagnostics. In high-burden scenarios, laboratories must be strategically located near outbreak hotspots to reduce turnaround times and improve testing capacity. Geographic information systems (GIS) can be used to map areas of need, helping to deploy resources where they are most critical. Additionally, collaboration with regional internal or external diagnostic hubs will ensure that smaller laboratories can send samples to better-equipped facilities for processing. This hub-and-spoke model can improve testing coverage for affected populations.

4.4 Supply Chain and Logistics Management

A reliable supply chain for test kits, reagents, PPE and other resources is vital. Efficient logistical systems must transport samples in a timely manner while preserving integrity. Stockpiling key materials and developing partnerships with global health organizations can manage sudden increases in demand. Robust supply chain systems are essential to ensure a steady supply of test kits and reagents, including buffer stocks and multiple suppliers to mitigate risks of shortages. Digital solutions to manage records can help streamline this process. While the demand might be lower in countries with a low disease burden, maintaining a reliable supply chain remains vital, and partnerships with neighbouring countries or international organizations can help maintain supplies.

4.5 Training and human Capacity Development

Substantial training and capacity development efforts are required. Healthcare workers and laboratory staff must be trained in technical and ethical aspects of mpox diagnostics. Continuing professional development is necessary to keep up with evolving testing technologies. In countries where there is wide community transmission of mpox, continuous training programs are necessary to keep up with the high demand and ensure accurate testing. In countries with sporadic mpox cases, periodic training sessions can help maintain readiness and ensure that healthcare workers are prepared for potential outbreaks. It is critical that all laboratory staff receive training on sample collection, sample referral and testing to ensure high standards of quality and to maintain biosafety measures.

4.6 Data Management and Reporting

Data management and reporting systems are crucial for tracking testing results and epidemiological data. Mpox testing must be integrated into existing health information systems to track results and monitor the spread of the disease. Digital tools can facilitate rapid reporting and enable real-time monitoring for epidemiological trends. Data sharing between countries will enable coordinated response efforts, however, it will be critical to always ensure data privacy.

4.7 Accessibility and Equity

Ensuring accessibility and equity in testing services is crucial. Testing services must be accessible to all, especially vulnerable populations. The use of mobile units and community-based sample collection or testing to reach all affected populations are important components of ensuring access to testing. In low-burden countries, accessibility efforts can be more targeted but should still ensure that remote and underserved areas are covered. Verbal consent and patient confidentiality must be maintained. Public health officials must ensure testing is voluntary and respects patient privacy.

4.8 Contingency Planning for Surge Capacity

Governments should prepare for rapid increases in testing demand by ensuring laboratories can scale up operations through activation of additional sites, deployment of more equipment and staff. Stockpiling test kits, reagents and PPE, and establishing rapid response teams are essential. Collaboration between national and regional health authorities can manage surge capacity effectively.

4.9 Community Engagement and Public Awareness

Public health education campaigns are necessary to inform the population about the importance of testing, symptoms and signs of mpox, and available services. Effective communication strategies that address local languages, customs and beliefs will increase testing uptake and reduce stigma. Cultural sensitivity and acceptability are important to address cultural beliefs and practices that may affect the acceptability of mpox testing. Engaging community leaders, stakeholders and specific communities or groups that might be at higher risk is essential to build trust, reduce stigma and increase acceptance to testing. It is important to empower communities to lead these efforts and implement strategies to counter misinformation by working closely with social media platforms to provide credible information.

4.10 Strategic Partnerships and Collaboration

Governments, NGOs, international organizations and private sector partners must collaborate to pool resources, expertise and funding. Partnerships with organizations like WHO and Africa CDC and other stakeholders are essential when planning and mounting a testing strategy. Collaborations with diagnostic manufacturers can secure a steady supply of test kits and equipment.

4.11 Funding and Resource Allocation

The cost of widespread testing can be substantial in countries with wide spread mpox transmission, necessitating sufficient funds from governments and international donors to subsidize testing and ensure affordability for all. Governments and health authorities must secure funding from multiple sources, including national budgets, international donors, and private foundations. Proper resource allocation prioritizes regions with the highest burden or greatest need for testing infrastructure.

5. SAMPLING STRATEGY AND SAMPLE TRANSPORT

5.1 Specimen collection

5.1.1 Collection in the hospital setting

In the hospital setting, specimen collection for mpox must follow strict infection prevention practices, including hand hygiene and the use of PPE. A viral swab in viral transport medium (VTM) collected from a mucosal or cutaneous lesion, such as an ulcer or vesicular fluid is the preferred sample. Dry swabs and lesions crusts may also be collected. If no lesions are present or during the prodromal stage, a viral throat swab may be taken even though the yield from such samples is very low. Follow-up samples are not typically required unless advised by the relevant national virology laboratory. The sample's anatomical site must be clearly labeled, and patient identifiers—such as full name or initials, date of birth, medical record number, and the requesting clinician's name—should be included. The specimen must be packed in a triple packaging, and the local microbiology or virology laboratory must be informed about the suspected case. Samples should be dispatched to the laboratory using courier services, avoiding the use of public transportation, and must be clearly labeled as **suspected mpox**

5.1.2 Collection in other settings

Specimen collection requirements are similar to those in hospital settings, including strict adherence to safety measures and proper labeling. Use a standard viral swab in viral transport medium for mpox testing. For international transport, specimens from suspected, probable, or confirmed cases of MPXV—including clinical samples, viral isolates, and cultures—must be classified as Category A (UN2814) infectious substance, affecting humans. This classification is essential due to the significant risk these specimens present. Although most MPXV materials are categorized as Category B (UN3373) infectious substances for transportation, viral cultures are specifically classified as Category A because of their increased risk of infection. Detailed guidance on preparing specimens for transport, including cross-border referrals, can be found in the Africa CDC cross -border specimen referral for laboratory diagnostic and genomic surveillance and the WHO guidance on regulations for the transport of infectious substances [18&19]

5.1.3 Specimen packing and transport

Provide instructions on where to ship the samples, based on national recommendations. If those do not exist, ask the designated reference laboratory in your country. Do not send samples without confirmation that they can be accepted and processed.

Delivery : The courier must be informed which package contains mpox samples so that they can notify the virology testing laboratory or referral laboratory upon delivery. Samples collected from suspected mpox cases should be clearly labeled as "probable/suspected mpox" and packaged by the laboratory following standard safety procedures.

Temperature: After specimen collection, store the specimens (dry swabs, swabs in VTM, lesion crusts) in sterile leak-proof containers. Use a durable container for the required shipping and temperature conditions. Glass containers are not recommended. If testing occurs at an external laboratory, contact the reference laboratory facility to determine specimen storage requirements. If specimen testing does not occur promptly after specimen collection, refrigerate (2-8°C) or freeze (-20°C or lower) in the local laboratory until testing occurs. Dry swabs, swabs in VTM, or

[18]Guidance on regulations for the transport of infectious substances, 2023–2024
[19]https://www.who.int/publications/i/item/9789240019720

lesion crust(s) that are stored at 2-8°C can be tested for up to 7 days from collection. Swab specimens in VTM and lesion crust(s) that are stored frozen (-20°C or lower) can be tested for up to 30 days from collection whereas dry swabs that are stored frozen (-20°C or lower) can be tested for up to 60 days from collection. It is important to consider longer term storage (-80°C) for future research and development work. Although mpox is a DNA virus, and DNA is more stable than RNA, samples should still be transported in temperature-controlled conditions, typically between 2 – 8°C (cold chain).[20] In some cases, DNA samples can be transported at ambient temperature if the sample is stabilized in special buffers (e.g., Lesion Swabs, Lesion Crusts).[21] For additional information regarding specimens shipped across borders, seek guidance in the Africa CDC cross -border specimen referral for laboratory diagnostic and genomic surveillance.

Triple packaging: Triple packaging is essential for the safe transport of mpox specimens, ensuring secure containment of potentially infectious materials.[21] It consists of three layers:

- Primary Container: A leak-proof tube or vial that holds the specimen.
- Secondary Packaging: A leak-proof outer layer that provides additional protection against leaks and contamination.
- Outer Packaging: A durable package that meets regulatory requirements (UN3373 Category B) for transporting biological materials.

Specimens should be packed according to the International Air Transport Association (IATA) requirements.[22] Key guidelines include clear labeling indicating the presence of infectious material, proper documentation detailing contents and sender/recipient information, and maintaining appropriate temperature conditions during transport. All handling should be conducted by dangerous goods-certified shippers, and personnel must wear appropriate personal protective equipment (PPE).[23]

Clear acceptance and rejection criteria: Laboratory personnel should record all specimens received in a laboratory accession book, worksheet, computer or comparable system, including the date and time of receipt of samples (turn-around-time), as well as the identity of the receiving officer.

Establish clear criteria for acceptance and rejection at the testing laboratory (e.g., checks on sample integrity, broken/leaking sample tube, specimen reaching the laboratory beyond 72 hours, inadequate sample volume depending on the sample type, etc.). The reason for sample rejection must be communicated back to the requesting clinic as part of corrective action and to initiate recollection of a specimen from the patient.

Note: *All cross-border specimen referrals should be accompanied by all relevant documentation including material transfer agreements (MTAs) and import/export permits where required.*

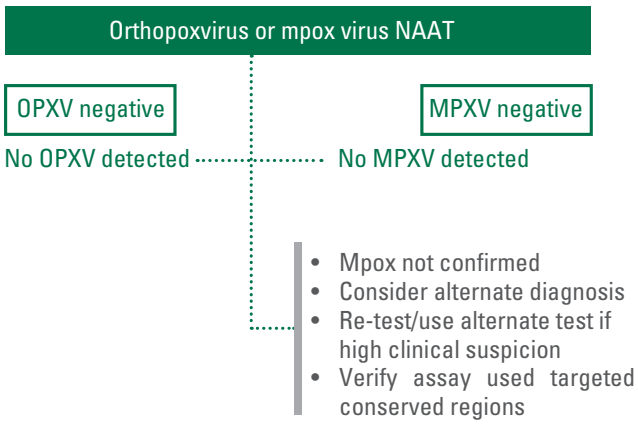
[20]https://www.cdc.gov/mpox/hcp/diagnosis-testing/collecting-specimens.html
[21]https://www.cdc.gov/locs/2022/06-23-2022-lab-advisory-CDC_Updates_Specimen_Collection_Guidelines_Monkeypox_Virus.html
[22]IATA (https://www.iata.org/en/programs/cargo/dgr
[23]https://www.who.int/publications/i/item/9789240019720

6. MPOX TESTING FOR PUBLIC HEALTH PURPOSES

6.1 Algorithm for case identification and testing

Every suspected mpox case should be offered testing (refer WHO case definition for mpox). The recommended specimen type for laboratory confirmation of MPXV is 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). Alternative specimen types, such as oropharyngeal swabs, can be collected in the absence of skin or mucosal lesions. However, such specimen types may provide less sensitive results for diagnosis than material from skin lesions. For this reason, a negative result should be interpreted with caution.[27-31] 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. [24-28]

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

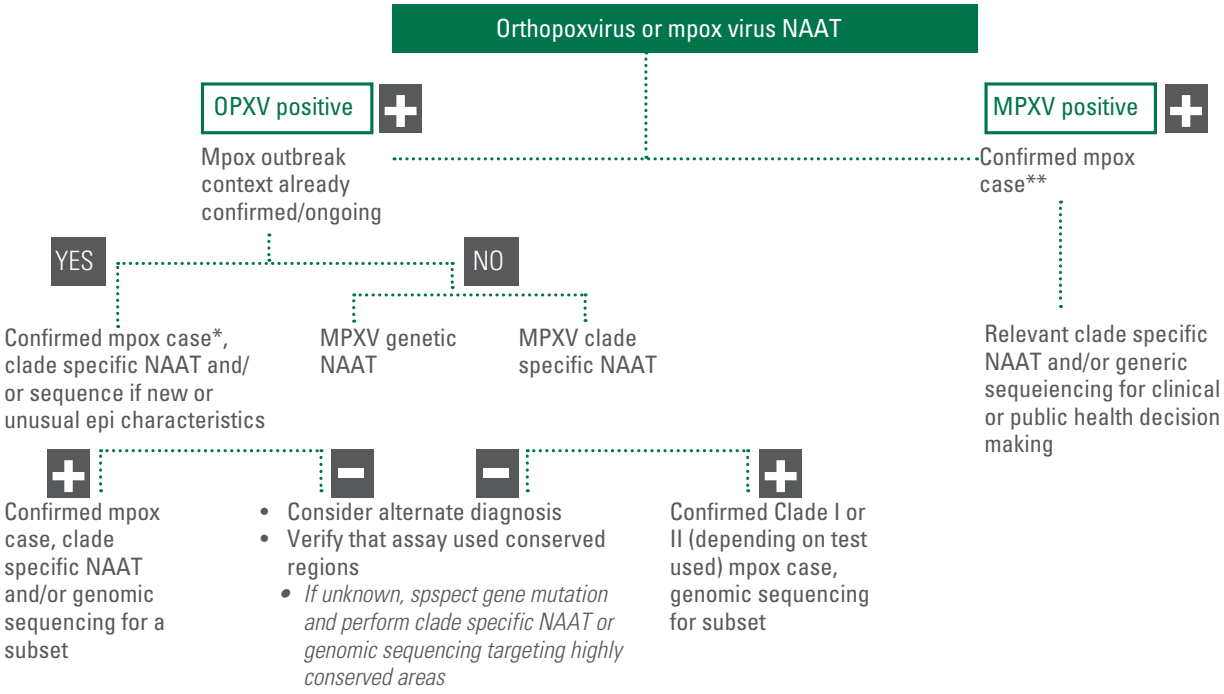


Note: *This testing algorithm for clinical management and surveillance of mpox: negative results is part of the WHO global lab guidance on mpox.*

Diagnostic testing for the monkeypox virus ("MPXV"): interim guidance, 10 May 2024

[24]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.
[25]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–
[26]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.
[27]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
[28]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.

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



Note: This testing algorithm for clinical management and surveillance of mpox: negative results is part of the WHO global lab guidance on mpox: positive results

Diagnostic testing for the monkeypox virus ("MPXV"): interim guidance, 10 May 2024

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. Positive detection using an OPXV PCR assay alone is generally considered sufficient for laboratory confirmation of mpox only in mpox confirmed settings and in settings with no other orthopoxviruses circulating in human populations (Figure 2).

A number of factors could contribute to false-negative results, such as poor quality of the specimen through poor sample collection, 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 to false negative results.[32] Positivity rate generally can be low if strict case definitions are not used to subject suspected cases for testing and this should be taken into consideration in test result interpretation.[33]

6.2 Alternative diagnosis and considerations for integration

As per the alternate diagnosis (Figure 2), other potential viral causes could include: 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 (cause of molluscum contagiosum), and rarely, tanapox. Other non-viral causes of rash in the differential diagnosis may include disseminated gonococcal infection (DGI), vasculitis, bacterial skin and soft tissue infections, medication allergies and chancroid. [29-31]

Prior infection with mpox or mpox vaccination does not guarantee full protection from future infection therefore suspected cases with repeat presentation should continue to be tested.[32-33] Currently, there are insufficient data on the usefulness or cost-effectiveness of screening for MPXV in asymptomatic individuals at high risk of infection.[30]

It is important to note that the differential diagnosis differs depending on the affected population. In children, which represent the highest proportion of clade Ia infections, chickenpox should be considered as the main differential diagnosis, and testing for those viruses should be done in case mpox test is negative. Additionally, measles testing should also be considered in such cases. As mpox cases are increasingly linked to sexual transmission, it is highly recommended that, in addition to mpox testing, any individual presenting with clinical symptoms suggestive of mpox contracted by sexual transmission is also tested for HIV and other STIs as indicated and subsequently receives appropriate medical care. Persons living with HIV who are immunosuppressed are at higher risk of developing severe mpox disease.[34] Therefore, most particularly where cases and outbreaks may be linked to sexual transmission or any circumstance where immunosuppression may be suspected or known to be present, patients with mpox for whom HIV status is not known should also be tested for HIV per the current WHO consolidated guidance on HIV testing services.[35]

6.3 Considerations for decentralized testing: algorithm, reporting systems and interpretation

Decentralization of mpox testing will consider the level of disease transmission and the laboratory infrastructure. The table below indicates the action to be taken in different disease transmission settings to adopt appropriate mpox testing strategy for optimal outbreak response

[29]World Health Organzation. Surveillance, case investigation and contact tracing for mpox (monkeypox). 2024 Mar 20; Available from: https://www.who.int/publications/i/item/WHO-MPX-Surveillance-2024.1

[30]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

[31]World Health Organization. Managing epidemics: key facts about major deadly diseases, 2nd edition. 2023 Nov 14; Available from: https://www.who.int/publications/i/item/9789240083196

[32]Li Z, Xia Y, Long J, Qi L. Mpox reinfection: A rapid systematic review of case reports. Infect Med (Beijing). 2024 Mar;3(1):100096.

[33]Musumeci S, Laflamme J, Kaiser L, Segeral O, Calmy A. Characteristics of possible mpox reinfection cases: literature review. J Travel Med. 2023 Nov 18;30(7):taad136.

[34]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/

[35]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.

Context /testing through-put (TP)	Recommendations for Testing	Key Actions for Optimization
No cases/low TP	▪ Test all suspected cases using laboratory-based NAAT. ▪ Testing should be performed by one or several nationally desig-nated reference laboratories. ▪ Sequencing of all positive cases in recommended.	▪ -Ensure referral and transport chain can support centralized testing ▪ -Strengthen or sustain capacity and exper-tise at the national public health laboratory ▪ -Establish a laboratory contingency plan including mapping of national testing resources and capacities and identify potential sources of infection (e.g. imported cases, zoonotic spillover events). ▪ -Prepare for the possibility of increasing transmission and plan for surge mpox testing capacity. ○ Revise relevant SOPs ○ Ensure a surveillance strategy is in place ○ Conduct simulation exercises
Sporadic cases /low (TP)	▪ Test all suspect cases. ▪ Testing should be performed by one or several nationally designated reference labo-ratories. ▪ Decentralization of labora-tory-based NAAT may be considered to sub-national laboratories with adequate technical capacities. ▪ Sequencing of all positive cases in recommended.	▪ Ensure referral and transport chain can support centralized testing and consid-er options for decentralized testing ▪ -Establish a laboratory contingency plan including mapping of national testing resources and capacities and identify potential sources of infection (e.g. imported cases, zoonotic spillover events). ▪ -Prepare for the possibility of increas-ing transmission and plan for surge mpox testing capacity. ○ Revise relevant SOPs ▪ Conduct simulation exercises
Clusters /moderate TP	▪ Test all individuals meeting the case definition. ▪ Consider sequencing at least 5% of all positive cases, any new introductions, suspected diagnostic failure, unusual clin-ical presentation, mpox case reported in non-endemic areas	○ Activate laboratory contingency plan in localized areas. ▪ Consider decentralization of testing using mPOC devices where appropriate

Context /testing throughput (TP)	Recommendations for Testing	Key Actions for Optimization
Widespread community transmission /high TP	▪ Test all individuals meeting the case definition to the extent possible. ▪ Consider sequencing at least 5% of all positive cases, suspected diagnostic failure, those with unusual clinical presentation, new introductions or first mpox cases in mpox non-endemic areas	○ Activate laboratory contingency plan. ▪ Consider expansion of testing capacity through the following: ▪ Activate localized surge capacity. ▪ -Expand localized testing facilities. ▪ Increase accessibility of testing facilities. ▪ Expand testing product options, including expanding the use of approved mPOC. ▪ Decentralize testing using all opportunities including integration with HIV and TB testing programs ▪ -Introduce mobile testing facilities. ▪ -Introduce mobile sampling facilities. ▪ -Deploy laboratory staff from other fields, including veterinary and academic laboratories, to support and provide resilience to mpox laboratory staff.

If decentralization strategies are implemented, reporting systems should converge to a single centralised information system to be used for data flow to the incident management system. Laboratories should follow national reporting requirements and be particularly attentive regarding confirmed cases with a relevant recent history of international travel..All MPXV test results, positive or negative, should be immediately reported to national authorities, Africa CDC and WHO.

6.4 Surveillance and testing for Mpox

To effectively manage Mpox outbreaks, a comprehensive surveillance and testing strategy is essential. This strategy should prioritize nucleic acid amplification tests (NAATs), particularly real-time polymerase chain reaction (PCR), as the primary method for confirming infections through clinical specimens such as lesion materials. Additionally, the development of rapid diagnostic tests can facilitate timely detection in various settings. A robust reporting system is necessary, including immediate reporting protocols for suspected cases and standardized data collection to monitor epidemiological trends. Integrating mpox surveillance with other infectious diseases will enhance resource utilization and improve public health responses, ensuring that healthcare providers are equipped to recognize and respond to multiple health threats effectively.

Africa CDC recommends enhancing surveillance within communities and health facilities and at the point of entries, linking these efforts to national and regional laboratories for confirmation

Case-based surveillance in health facilities: The national level should adopt the standard WHO case definition for mpox and disseminate this definition to all facilities to facilitate detection and reporting. In addition, responsible healthcare workers should use the standardized line list to inform case-based data collection efforts in health facilities. This ensures standardized and comparable case reporting across AU Member States.[36]

Cased-based surveillance in the community: In countries where event-based surveillance (EBS) is not in place or in areas where community health workers have not been trained on EBS, simplified case definitions could be used to help identify cases. Simplified community case definitions could also be used in areas with established community mpox trans- mission to improve active case finding efforts in communities.[36]

Testing in Primary Healthcare Settings within the framework of Integrated Disease Surveillance and Response (IDSR)

Context of IDSR in Mpox Surveillance: Primary healthcare (PHC) settings are essential in the context of Integrated Disease Surveillance and Response (IDSR) for detecting, diagnosing, and responding to mpox outbreaks. IDSR promotes a coordinated and standardized approach to disease surveillance at all levels of the health system, including PHCs, which serve as the frontline for community health interventions. Integrating mpox testing into PHC through the IDSR system will ensure that cases are rapidly identified, appropriately managed, and reported in a timely manner, facilitating an effective outbreak response.

Sample Collection and Laboratory Referral:

PHCs should be equipped to collect lesion swabs, oropharyngeal, or rectal swabs for laboratory confirmation using PCR. Under IDSR, all collected samples should be referred to designated laboratories as per national guidelines. A streamlined sample referral system needs to be established to ensure that collected samples from PHCs are transported to testing facilities efficiently, particularly in remote areas. This should be coordinated within the IDSR structure, where public health laboratories form part of a well-defined laboratory network.

Data Harmonization and Real-Time Reporting:

Testing results, including both confirmed and suspected cases, should be reported in real-time through national IDSR platforms to facilitate timely decision-making and outbreak management. PHCs must have tools, such as mobile or web-based reporting systems, that are aligned with IDSR reporting standards. Data from PHCs should feed into national databases, ensuring harmonization across districts and regions, which is critical for tracking outbreak trends and coordinating response efforts.

Cross-Border Surveillance and Points of Entry (PoE):

Surveillance at points of entry: Africa CDC recommends surveillance at all points of entry (POEs) including air, land and sea. Enhanced screening at PoEs can help identify travel-related cases.[36]

PHCs located near borders or major travel hubs should implement surveillance that aligns with IDSR protocols for points of entry. This will ensure that suspected mpox cases are detected early, especially those with travel history or exposure to areas with known outbreaks. Standardized reporting mechanisms for cases identified at borders should be integrated into PHC systems to ensure that these cases are appropriately managed and followed up.

Implementation of Mpox Testing in PHC Settings

To successfully implement mpox testing in PHC settings as part of the IDSR framework, the following steps should be taken:

1. **Training and Capacity Building**:

- Conduct regular IDSR training for PHC staff, focusing on mpox case definitions, sample collection, syndromic surveillance, and the use of real-time reporting tools.
- Train CHWs and other community actors in early detection, contact tracing, and the reporting of suspected mpox cases within the IDSR framework.

[36]https://africacdc.org/download/mpox-surveillance-reporting-protocol-for-african-union-member-states/

2. **Resource Allocation**:

- Equip PHCs with appropriate diagnostic tools, such as point-of-care (POC) testing kits for rapid mpox screening. Where POC testing is not feasible, ensure that facilities have the skills and materials needed for proper sample collection and transportation.
- Strengthen the logistics system to facilitate timely sample transport from PHC settings to designated laboratories for confirmation testing.

3. **Use of IDSR Tools for Data Collection and Reporting**:

- Integrate mpox surveillance into existing IDSR tools and platforms at the PHC level, ensuring that all suspected cases, test results, and epidemiological data are captured and reported.
- Utilize digital tools to enhance the speed and accuracy of data collection and reporting, ensuring that PHCs can input data directly into IDSR databases.
- Countries are encouraged to share the case-based data with the African CDC and the WHO using the validated reporting tools.

4. **Community Engagement and Risk Communication**:

- Engage community leaders, civil society organizations, and local health advocates in the dissemination of information about mpox symptoms, preventive measures, and when to seek testing. This will help to ensure that cases are detected early and reported through community-based surveillance systems linked to PHCs.
- Use local languages and culturally relevant messaging to increase awareness and reduce stigma around mpox testing and reporting.
- Incorporate use of appropriate media channels that readily accessible to the affected population

5. **Collaboration and Coordination**:

- Establish clear communication channels between PHCs, district health teams, and national surveillance units to ensure coordinated efforts in outbreak detection and response.
- Collaborate with neighboring countries to harmonize cross-border surveillance efforts, especially in regions with frequent travel and trade.

6. **Monitoring and Evaluation**:

Regularly review the performance of PHC-based mpox testing and reporting within the IDSR system. Key performance indicators (KPIs) should include the number of

- suspected and confirmed cases, turnaround time for testing, and the timeliness of case reporting.
- Evaluate the integration of mpox surveillance into IDSR regularly to identify gaps and improve the overall efficiency of the surveillance system.

7. SEQUENCING AND GENOMIC SURVEILLANCE

The diagnosis of Mpox relies on real-time PCR testing of skin lesion materials, but whole genome sequencing (WGS) of confirmed cases is essential for clade typing, which helps in characterizing outbreaks, tracking the spread of clades, and informing response strategies. Africa is a key region for MPXV genetic diversity, with both clade I and clade II, and their subclades, circulating widely. Enhancing and systematic mpox sequencing in Africa is crucial due to the virus's potential for genetic changes, particularly through human-to-human transmission, which introduces mutations such as APOBEC3-driven changes. These genetic alterations, combined with epidemiological and clinical data, provide vital insights for managing outbreaks. The mutational potential of MPXV, including large deletions affecting diagnostic assays, underscores the need for robust genomic surveillance and research to monitor the virus's evolutionary dynamics and its implications for public health and global health security. Member States facing intense transmission of MPXV should incorporate routine genomic sequencing into their surveillance strategies. This approach entails systematically sampling at least 5% of confirmed cases to reflect the temporal, geographical, and epidemiological aspects of the outbreaks. Additionally, genomic sequencing can be utilized to monitor the spread of the virus within the country.[37-39]

7.1 Mpox genomic surveillance

Strengthening laboratory capacity for NGS and bioinformatics analysis is crucial. More investment is needed to build laboratory infrastructure, provision of supplies and training of laboratory personnel in specimen collection, handling, and sequencing to implement mpox genomic surveillance. The primary objectives of mpox genomic surveillance are:

- To track the genetic evolution and spresd of the Mpox virus in real-time.
- To identify and monitor emerging clades that may impact disease transmission, severity, or vaccine effectiveness.
- To inform public health decision-making, including outbreak response, contact tracing, and prevention strategies.

Viral DNA is extracted from the collected specimens and subjected to whole-genome sequencing using next-generation sequencing (NGS) technology. The generated sequence data is then analyzed to identify mutations, variants, and phylogenetic relationships. Sequence data should be shared with relevant public health authorities and international databases like GISAID. Collaboration with regional and global networks facilitates data analysis and interpretation. Bioinformatics tools can be used to track the emergence and spread of mpox variants. The genomic data is interpreted in the context of the epidemiological situation. Findings are communicated to public health officials, healthcare providers, and the public. Guidance on potential implications for disease transmission, severity, and vaccine effectiveness is provided. Integrating genomic surveillance data in to broader public health surveillance and response efforts and establishing a coordination mechanism for genomic surveillance at regional and national levels are very critical.

[37] https://africacdc.org/download/mpox-sequencing-in-africa/
[38] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10783113/
[39] https://www.sciencedirect.com/science/article/pii/S0264410X24001257

8. QUALITY ASSURANCE OF MPOX TESTING AND SEQUENCING: KEY CONSIDERATIONS

Errors in diagnosis can lead to inappropriate treatments or wasted resources. Accurate identification, especially during outbreaks like MPXV, is crucial, with nucleic acid-based amplification testing (NAAT) being the current standard. However, many laboratories in resource-constrained settings lack proper accreditation and quality management systems (QMS), increasing the risk of errors. This guidance outlines essential measures for minimizing laboratory errors during testing for MPXV, with a focus on the entire testing process and key quality assurance (QA) requirements.

8.1 Pre analytical

The pre-analytical phase encompasses all steps needed from obtaining specimens from the patient to the analytical assay, including specimen collection and handling prior to laboratory receipt. Significant errors can occur during this phase, particularly related to specimen handling and identification. Therefore, rigorous control measures are essential to prevent issues from propagating downstream. Key considerations for this phase include sample collection, packaging and shipping already highlighted in section 5. In addition, quality considerations in procurement of equipment, test kits and reagents and PPEs are critical

Procurement of equipment, test kits, reagents and PPE

- Laboratories and partners able to procure diagnostics are advised to only procure those listed in the Africa CDC Diagnostics Advisory Committee (DAC) second edition of recommended RT-PCR tests for mpox.[40] those with emergency use authorization from the United States Food and Drug Administration[41] or those in the WHO's emergency use list.
- Procurement of supplies is best done through existing distributors or supply networks within the country. Due consideration should be given to forecasting and procurement of ancillary reagents (e.g. extraction buffers and sample collection materials).
- Reagents and kits should be shipped and stored at the temperatures recommended by the manufacturers.

8.2 Analytical Phase

The analytical phase involves the actual laboratory testing processes and procedures that yield diagnostic results, with availability of PCR and sequencing SOPs being essential.

In-house Assay Validation and Verification

Before introducing a new test, laboratories must ensure it performs as intended. This process involves either validation or verification. Validation is a rigorous procedure that establishes test performance, typically conducted by the test developer or manufacturer and often required for regulatory approval. Verification, on the other hand, confirms that the test continues to meet performance standards established during validation and must be done before offering the new test. Commercially validated assays require only verification by the user, while non-standard or modified methods need full validation. Less experienced laboratories can seek mentorship from more experienced laboratories to confirm their initial test results and improve performance and can perform on-going verification using split sample testing with a reference laboratory or using established methods.

[40] https://africacdc.org/download/mpox-molecular-diagnostic-testsrt-pcr-2/
[41] (https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices,

Reagent Preparation

Commercial kits usually include all necessary reagents, but it is important to check for any additional requirements, such as extraction reagents. These should be reconstituted in a biosafety cabinet or laminar flow hood, following the product instructions and ensuring the correct temperature conditions. Freeze-thaw cycles should be minimized, and reagents must be labeled with the date they were received and opened, along with the initials of the responsible laboratory personnel. Expiration dates must always be checked, and expired reagents should not be used. Reagents from different test kit lots or manufacturers should not be mixed, and pipette tips should be changed between all manual liquid transfers. It is important to maintain separate areas for assay setup and nucleic acid handling, and primers and probes should be handled with extra care, and aliquoted for single-run use after resuspension and dilution.

Sample Processing

DNA extraction must be performed in a biosafety cabinet within a BSL-2 or equivalent facility. To avoid repeated freeze-thaw cycles, specimens should be aliquoted into separate tubes, and must be allowed to thaw completely before use, ideally on cold blocks or ice. Test tubes must be clearly labeled with specimen details to ensure traceability, and pipettes with aerosol-barrier or positive-displacement tips should be used. New pipette tips should be used for each specimen to avoid cross-contamination.

Library preparation is a critical step in sequencing workflows, where DNA or RNA is processed into a format suitable for high-throughput sequencing. Ensuring quality at this stage directly impacts the success of downstream sequencing, data quality, and overall interpretation of results. Effective quality control (QC) during library preparation can prevent errors and artifacts and ensures that sequencing is both accurate and cost-effective. QC checks must be implemented to ensure nucleic acid integrity, proper fragmentation, adapter ligation efficacy, amplification (where applicable), correct size distribution and quantity and absence of contaminants that could affect sequencing efficiency.

Quality Control

Quality control (QC) is a critical aspect of the analytical phase, involving the examination of control materials or known substances/reference datasets (for bioinformatics analysis) or non-template controls alongside patient specimens. QC ensures the accuracy and precision of the entire analytical process. Each run must include QC measures that cover every critical step of the PCR analysis to monitor performance effectively. Bioinformatics pipelines to analyze genomic sequencing data should be developed or adapted using best practices and validated before use. Use of standard pipelines for virus assembly is highly recommended. Where in-house methods are used, the entire audit trail should be preserved for reproducibility, including sequence raw files.

8.3 Post-analytical phase

The post-analytical phase is the final phase of the total testing process and involves evaluation of laboratory test results; release of test results in a timely manner to appropriate individuals, particularly critical results; and modification, annotation or revocation of results as necessary to support clinical decision-making. The key processes under this phase include:

Test result interpretation and use for patient management

- Result interpretation should follow available guidance or algorithms.
- If the result is discordant, the patient should be resampled and possibly the sample sequenced.
- Any surprising result should be sent for confirmation at an international reference laboratory.

Test result reporting

- Test results must be reviewed independently by a laboratory supervisor to confirm accuracy before they are released to the requesting clinician. This involves confirming that the patient details are correct and matching the test requisition and validity of the test indicated by the control results.

Notification for disease surveillance

- Laboratories should follow national reporting requirements. All tests whether positive or negative should immediately be reported to national authorities.

8.4 External Quality Assessment

EQA allows a laboratory's testing performance to be compared to the performance of a peer group of laboratories. The three different methods for EQA programs are described below.

Proficiency testing (PT): an external provider sends a blinded, well-characterized panel at intervals (usually quarterly) to a set of laboratories for analysis. The blinded panel is treated like a patient sample during testing, and the results are analyzed, compared and feedback reports generated. Laboratories should choose providers experienced in delivering PT in their region.

CDC Proficiency Testing:The Centers for Disease Control and Prevention (CDC) often conducts PT programs for laboratories testing for various infectious diseases, including MPXV. They provide blinded samples for laboratories to test, along with detailed feedback on their performance.

NIST PT Programs: The National Institute of Standards and Technology (NIST) may offer PT programs that include emerging infectious diseases. They provide standardized samples that laboratories can use to ensure their testing methods are accurate.

WHO Laboratory Quality Assurance: The World Health Organization (WHO) occasionally organizes PT schemes for member states, particularly for emerging pathogens. They may provide PT panels for laboratories testing for MPXV, including specific criteria and performance metrics. The logistics are managed by INSTAND (EQA service provider) & WHO Country Offices (WCO). As consignees for distributing panels to participating laboratories within their own countries.

Laboratories should choose providers experienced in delivering PT panels within their region.

Rechecking or retesting: samples tested by one laboratory are retested by another laboratory (inter-laboratory comparison). Rechecking can be employed in the absence of a PT program.

On-site evaluation: usually done in addition to PT or rechecking and may be done when it is difficult to conduct traditional PT or rechecking and retesting. An evaluator (e.g. staff from the national reference laboratory) will visit the laboratory to check if the laboratory is meeting quality requirements, will retest and verify a few test results and provide advice to correct any faulty procedures. On-site visits are also important to motivate staff and provide refresher training if needed.

Table- 1: Challenges in implementing QC and possible solutions

Challenges	Mitigation measure
Unavailability of controls	Positive control: use of a confirmed positive patient sample. Negative control: use water/universal transport media/viral transport media.
Most methods are under development hence no validation data	Use methods with emergency use listing by WHO and second edition of recommended RT-PCR tests for mpox by Africa CDC throuhg its Diagnostic Advisory Committee. The list will be updated regularly as the DAC reviews evidence on RT-PCR tests for mpox.
Unavailability of PT schemes	Develop inter-laboratory comparison and send positive samples to national reference.
Development of genome sequencing and bioinformatics SOPs and documentation	Genomics and bioinformatics are relatively new methods in public health. To develop SOPs for genomic sequencing and bioinformatics and other documents to address various quality essentials, laboratories can use templates.[42]

9. SAFETY AND ETHICAL CONSIDERATIONS

Healthcare personnel, persons working with wild animals or those working in veterinary laboratories are at risk from occupational exposure to orthopoxviruses. Health workers may be exposed during the care of patients with suspected or confirmed cases of mpox, during administration of certain vaccines, during sample collection and packaging, and during sample processing in the laboratory. Although the quantity of virus in blood and body fluids is low, specimens from skin lesions (swabs from lesion surfaces, pus and exudate, and lesion crusts) are likely to contain high quantities of monkeypox virus. Samples from both clade I and II MPXV are designated as Category B infectious substances, and therefore all clinical specimens must be appropriately handled. Since infections resulting from occupational exposure to the monkeypox virus (MPXV) can be serious, all those at risk must adhere to recommended biosafety measures. Safety measures include the following:

- Pre-exposure vaccination with a vaccine containing a non-replicating (MVA-BN) or minimally replicating strain of vaccinia virus; ACAM2000 which contains a replicating strain may also be considered. MVA-BN is given as two doses at least 28 days apart, and ACAM2000 are given as a single dose. Booster doses given every 2–5 years may be required if there is ongoing risk of exposure.[43-44] Contraindications to receiving vaccines include atopic dermatitis, vaccine component allergy, pregnancy or breastfeeding, immunosuppression, and major underlying heart disease; however, MVA-BN may be considered in pregnant and immunosuppressed individuals. Persons who have recovered from mpox do not require vaccination.
- Routine diagnostic specimen processing should be conducted in Biosafety Level 2 (BSL-2) laboratory facilities.[45] Specimens should be processed in a Class II Biosafety Cabinet (BSC), especially if there is a potential to generate aerosols. Additional precautions to reduce risk and protect the skin and mucous membranes of the eyes, nose, and mouth include:

[42]https://cdc.gov/lab-quality/php/ngs-quality-initiative/qms-tools-resources.html
[43]World Health Organization. Smallpox and mpox (orthopoxviruses) vaccine position paper. Weekly Epidemiological Record. 23 August 2024, 99th YEAR No 34, 2024, 99, 429–456 http://www.who.int/wer
[44]Rao AK, Petersen BW, Whitehill F, et al. Use of JYNNEOS (Smallpox and Monkeypox Vaccine, Live, Nonreplicating) for Preexposure Vaccination of Persons at Risk for Occupational Exposure to Orthopoxviruses: Recommendations of the Advisory Committee on Immunization Practices — United States, 2022. MMWR Morb Mortal Wkly Rep 2022;71:734–742. DOI: http://dx.doi.org/10.15585/mmwr.mm7122e1

- Solid-front gowns with cuffed sleeves
- Double gloves
- Eye protection (safety glasses, snugly fitting goggles) or face protection (face shield)
- Particulate respirator equipped with N95 filters or higher.
- Limiting the number of laboratory personnel where specimen manipulation is conducted.
- Where centrifugation is required for a procedure outside a BSC, safety cups or sealed rotors should be used.

Laboratories regularly handling suspected or confirmed mpox materials should perform a site-specific and activity-specific risk assessment to identify and mitigate risks. The measures put in place depend on the following:

- The procedures performed.
- The hazards involved in processes and procedures.
- The competency level of the personnel performing the procedures.
- The laboratory equipment and facility
- The resources available
- The vaccination status of the personnel performing the procedures.

Decontamination and waste management

Hospital-grade disinfectants are suitable for routine cleaning, disinfection and decontamination procedures. The manufacturer's directions for concentration, contact time, and care and handling should be followed. Reusable PPE should be cleaned and disinfected according to manufacturers' instructions because some disinfectants may degrade the PPE.

Sharps should be disposed of in appropriate puncture-resistant containers, placed in a secure waste holding area, and incinerated. All residual specimens and monkeypox virus waste should be decontaminated before incineration. Materials intended for disposal outside the immediate facility should be placed in durable, leak-proof containers and securely closed for transport. Facilites should follow local, regional, state, national and international regulations for waste disposal.

Ethical issues

Due to populations at high-risk for mpox, diagnostic approaches for mpox may be subject to discrimination and stigmatization. Testing must therefore be conducted in a respectful and responsible way, ensuring risks to persons are minimized and collaboration of all involved parties.[46] Ethical approaches include minimization of risks of exposure and data protection through strict confidentiality, and ensuring public cooperation through providing information about the disease and how data will be used. It is essential to demonstrate a commitment to responsible practices that prioritize the well-being of individuals and the community.

[45]Centers for Disease Control and Prevention. Biosafety Laboratory Guidance for Handling and Processing Mpox Specimens. April 4, 2024.
[46]WHO guidelines on ethical issues in public health surveillance. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.

10. MONITORING AND EVALUATION OF MPOX TESTING STRATEGIES

Monitoring and Evaluation (M&E) is critical to track progress and assess effectiveness of implementing the mpox testing strategy. The KPIs for the Mpox Testing Strategy. (Annex -2) provides an outline of important M&E metrics that countries are encouraged to adapt to their local needs. It includes key performance indicators (qualitative and quantitative process and output) that align with objectives of the strategy. Member states can use the data to measure their progress against implementation of the strategy and support decision making for testing including testing capacity and resource allocation.

ANNEX

Annex-II The Proposed KPIs

Strategy objective	Purpose/rationale	Measure	Target	Numerator/denominator for quantitative measures	Data source	Timeframe for reporting	Reporting frequency
Implement widespread testing in symptomatic individuals	To contribute towards accurate surveillance and epidemiology including changes to the outbreak	Proportion of tests performed on reported cases	80%	Numerator: Number of suspected cases tested Denominator : Number of suspected cases reported	Aggregated national data	Weekly	Weekly
Support the surveillance system that includes routine testing in high-risk areas and provide testing options and sampling strategy and sample transport for prompt testing to recognize and manage the disease.	Timely sample collection and receipt in the laboratory improves quality of the sample and contributes to result accuracy	% of referred samples with result received within the TAT Sample collection to sample receipt in laboratory Sample receipt in laboratory to sample result release	Defined by the referral network	The numerator: the total number of referred samples for which results were received within the specified TAT The denominator : the total number of referred samples during the same reporting period	Result log book	Weekly	Monthly
Collect and analyze data on testing outcomes, effectiveness, and trends to inform public health responses and refine testing strategies.	Positivity rate will depend on assay performance, prevalence of disease in the community and case selection for testing This measure cannot be used in isolation but may be useful for comparison between different scenarios/countries/assays	Positivity rate (outcome) Number of positive tests	Determine by the country	Numerator = number of positive tests Denominator = number of tests performed	Laboratory log book	Weekly	Bi- weekly

Strategy objective	Purpose/rationale	Measure	Target	Numerator/denominator for quantitative measures	Data source	Timeframe for reporting	Reporting frequency
Implement quality control measures for testing processes, and regularly review and update testing protocols based on the latest scientific evidence and technological advancements.	Laboratories performing Mpox diagnosis should have robust quality assurance processes in place. Including EQA to provide assurance of accuracy of the laboratory result	Evidence of participation in Mpox EQA scheme that complies with international quality standards - Specimen rejection rate % of rejected samples	Yes/No	Numerator: total number of testing facilities enrolled in EQA and scored acceptable results Denominator: Total number of testing facilities enrolled in EQA program	Individual laboratorial Sample reception register log book Sample reception register log book	Bi- annually Quarterly Monthly	Annually Quarterly Monthly
	The QC failure rate may highlight issues with assay performance related to the assay or the performer of the assay	Number (%) of QC failures/ invalid results (process)		Numerator = Total number of samples rejected Denominator = Total number of samples received Numerator =Total number of invalid test runs due to QC failure Denominator = Total number of test runs overall	QC log book	Monthly	Monthly

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