Considerations for wastewater and environmental surveillance for monkeypox virus

Interim guidance
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World Health Organization

Key points

Summary

Wastewater and Environmental Surveillance (WES) has potential to provide actionable information as part of multimodal surveillance for mpox. Use cases are:

- Early detection, as well as reassurance of the absence, of transmission to enable targeted risk communication and responses.
- Differentiation of circulating virus clades subject to validation and optimization of clade specific laboratory methods.
- Quantification of trends particularly in sewered and moderate to high case load settings if research further validates consistent correlation with cases.

Case-based surveillance for mpox remains the priority and any complementary WES for monkeypox virus (MPXV) must be integrated as part of multimodal surveillance. Technical and operational feasibility of WES has been demonstrated in sewered settings and to a lesser extent in unsewered settings. Cost-efficiency may be optimized though integration with existing WES for other targets (e.g., polioviruses or SARS-CoV-2). Ethical and legal aspects should be considered, including in relation to vulnerable or marginalized population groups. Further research is needed to outline best practice and develop recommendations on WES for mpox.

Definition and purpose

- WES is infectious disease surveillance using samples from sewage, or other environmental waters which include human wastewater.
- Mpox is an infectious disease caused by different strains of MPXV grouped in clades I and II and subclades Ia, Ib, IIa and IIb.
- This document provides information and key considerations for the use of WES for detection of MPXV as part of integrated multi-modal mpox surveillance and response.

Mpox is an emergent global health threat

- A Public Health Emergency of International Concern (PHEIC) for Mpox was declared from July 2022 to May 2023 due to multi-country spread of MPXV clade IIb and a further PHEIC was declared in August 2024, principally due to an upsurge of cases in the Democratic Republic of the Congo and spread of newly identified clade Ib, and the presence of all subclades in the African region and of IIb globally.
- Clade Ia MPXV is enzootic in multiple sub-Saharan African countries with unidentified animal reservoirs and recurrent spillover events which lead to secondary transmission of mpox in clusters and outbreaks.
- Since 2022, substantial clade Ia outbreaks have occurred in the Democratic Republic of the Congo, with the more recent identification of genotypically distinct clade Ib which is also characterized by sustained human-to-human transmission through both sexual and non-sexual routes. Based on limited evidence, clade 1b mortality continues to be lower that clade 1a.
- Clade IIb transmission also continues globally, predominantly involving men who have sex with men with spread through sexual contact.

Public health use cases of WES for MPXV

WES for MPXV may provide actionable information for mpox in the context of the current response. WES and case-based information are complementary and should always be considered together. WES may strengthen surveillance as case-based data may not be complete or timely, particularly if there may be cases not diagnosed or paucisymptomatic or pre-symptomatic transmission. MPXV WES objectives must be contextual with local relevance.. Objectives may include:

- Early detection, as well as reassurance of the absence of substantial community transmission
- Differentiation of circulating virus clades from WES
- Quantitative trends and additional genomic characterization from WES, if validated through further research, could also provide useful actionable information for mpox response.

Technical feasibility of WES for MPXV

- MPXV WES PCR detections concurrent with earliest clinical cases in multiple sewered settings, as well as early detections in absence of reported cases in unsewered settings, have suggested technical feasibility of WES for clade IIb MPXV. Interpretation of results must consider limitations including the likelihood of possible false negative results.
- Direct evidence is required to confirm clade IIb WES methods can also be applied to clade Ia, Ib and IIa.
- Validation of PCR use and sequencing methods to differentiate subclades is required.

Operational feasibility of WES for MPXV

- Operational feasibility has been demonstrated at scale in multiple settings for routine as well as agile, responsive WES programs for MPXV clade IIb including as part of multi-target WES. Experience has come from predominantly sewered settings with limited experience in non-sewered settings.

Ethical and legal considerations

- Consideration of ethical issues is critical for any surveillance, including WES. Considerations may differ by context and need for protection of groups who may be at risk of stigma and in difficult social or legal contexts such as men who have sex with men, sex workers and other vulnerable or marginalized groups most affected by mpox. Principal considerations include privacy, data sharing, program effectiveness, potential for harm and approaches to mitigate harm.
- Legal and regulatory considerations are also relevant; including those related to ownership of samples and data, and other regulatory requirements encompassing the occupational health and safety of workers involved in WES sampling and analysis.

Integration and cost-effectiveness

- Integrated mpox program - WES and other surveillance streams should be designed to optimize cost-effectiveness so that information is rapidly combined to inform actions in the mpox response.
- Multi-target WES - If MPXV WES is implemented, existing WES (such as for poliovirus, SARS-CoV-2 or other) should be leveraged wherever sampling, analytic, reporting and other processes can be aligned.

Knowledge gaps

Strategic and applied research is recommended to rapidly fill knowledge gaps and expand the utility of WES particularly in relation to;

- Optimizing sampling and analytic methods for clade Ib MPXV, including in unsewered settings with interpretation of results to strengthen overall surveillance and response.
- Evaluating and optimizing positive and negative predictive values for mpox in African settings at the highest risk with timely accurate differentiation of clade Ib from other subclades.

Special use case for global sentinel surveillance at air transport hubs

- A globally representative WES system with sentinel air transport hubs implemented in collaboration with the aviation sector may detect circulation of pathogens (including mpox subclades) providing early warning and additional intelligence while promoting global equity.
- Reporting of WES for MPXV results to WHO is encouraged in accordance with IHR obligations.

1. Introduction

1.1. Purpose

The purpose of this considerations document is to provide globally applicable advice on the following questions in the context of the current mpox PHEIC:

- Why and how could WES for MPXV add value to public health decision making in different settings and contexts? (Sections 4 and 5)
- What are the current evidence and knowledge gaps on the technical and operational feasibility of WES for MPXV for all subclades in setting with and without sewered sanitation systems? (Sections 2, 3 and 8)
- What are practical and ethical considerations for WES for MPXV? (Section 6)
- How can integration of WES with other mpox surveillance and response and with any other WES activities be optimised ? (Section 7)

1.2. Target audience

This document is targeted at public health officials, mpox incident management teams and WES practitioners considering integration of MPXV WES, into mpox surveillance and response strategies. It also identifies key knowledge gaps. WES practitioners include multiple disciplines from the health and water and sanitation sectors. This document is intended to:

- assist decision makers, policymakers and public health professionals in making informed, evidence-based, ethical decisions on the value of WES for MPXV in their context to help decide whether and how to implement such a programme;
- support integration of MPXV WES surveillance as part of the overall mpox surveillance and response as well as the integration of MPXV WES with other WES activities insofar as these are synergistic and cost-efficient;
- promote sharing of mpox WES methods, approaches and applied research to fill knowledge gaps and advance evidence-based applications;
- guide utilisation of mpox WES results along with other mpox surveillance modalities in means of public health decision making; and
- support sharing of lessons and case studies from implementation experiences for more efficient application of WES globally for emerging health threats.

1.3. Scope

WES is surveillance using samples from sewage, or other human-wastewater impacted environmental waters. Sampling from the latter may be of relevance in locations where there are no or dysfunctional sewered sanitation systems. In relation to WES, such human-wastewater impacted environmental surveillance is reduced in scope from all environmental surveillance (i.e. not air, soil or other environmental samples but inclusive of wastewater derived sludge or solids).

This document provides information and key considerations relevant to the use of WES for MPXV as part of integrated multi-modal mpox surveillance and response.

2. General information

2.1. MPXV and mpox disease

Mpox is a disease caused by infection with monkeypox virus (MPXV). MPXV is an enveloped, double-stranded DNA virus of the genus *orthopoxvirus* within the family *Poxviridae*. Orthopoxviruses include variola virus, which causes smallpox. MPXV was first described in a captive colony of primates in 1958 and as a cause of human mpox disease in the 1970s *[1, 2 3]*.

MPXV is a zoonotic pathogen with animal reservoirs, likely in multiple mammal species within Africa. A wide variety of mammals including non-human primates (not limited to Africa) are potential hosts and vectors *[4, 5, 6, 7].* Currently there are no known reservoirs outside of Africa.

The disease in humans is clinically similar to smallpox, including distinctive skin lesions and fever. It is generally milder and less transmissible than smallpox but has been associated with severe illness and deaths among immunocompromised persons. Mode of transmission and viral clade also influence disease presentations. The incubation period from the time of exposure to the onset of symptoms can last from 2 to 21 days, though it typically ranges between 7 and 14 days *[8, 9].* Viral shedding and transmission may also occur in the absence of, or prior to symptoms *[10, 11, 12, 13]*.

Common symptoms of mpox are prodromal period with fever, headache, muscle aches, back pain, low energy, and swollen lymph nodes followed by an eruptive skin rash or mucosal lesions which can last 2–4 weeks. Of the two MPXV clades, clade I (now clade Ia) appears to have a higher case fatality rate (CFR) compared to clade II *[14]*. However, estimated clade Ia CFR is skewed by under-reporting of less severe cases and reliance on suspect case numbers without laboratory confirmation. In recent years, there has been an increase in mpox cases within Africa *[15].*

The recently described subclade Ib is distinct from subclade Ia *[16, 17, 18]*. Clade Ib appears from preliminary data to be less severe than subclade Ia with clinical presentation influenced by mode of transmission *[19].*

2.2. Global burden and distribution

Since the eradication of smallpox in 1980, mpox has emerged as the most important orthopoxvirus for public health. Prior to the 2022 outbreak, almost all mpox cases in people outside of Africa were linked to international travel or through imported animals from enzootic areas *[4, 20, 21, 22]*.

In 2022, a global multi-country outbreak of MPXV clade IIb was declared a PHEIC on July 23, 2022. A rapid increase in cases with global spread resulted in a rapid peak and decline with the first PHEIC ending in May 2023. The most cases by a single country were reported by the US-CDC *[23]*.

Since 2023, the Democratic Republic of the Congo has had a substantial MPXV outbreak with the more severe Clade Ia *[14]* and unprecedented case numbers and deaths, most of which were not laboratory confirmed. A recently identified clade (named as clade Ib) *[24]* with characteristic genetic mutations was detected in the eastern region before spreading to the capital Kinshasa *[17, 18, 25]*. Recent upsurge of cases and spread of clade I to other parts of the Democratic Republic of the Congo as well as to other African countries *[26]*.

2.3. Demographics of at risk and high risk populations

Prior to 2022, mpox cases and outbreaks occurred almost exclusively in populations living in forested, rural areas in West and Central African countries, where the virus is enzootic. Sporadic cases and clusters occurred elsewhere with spillover human transmission with limited onward human-to-human spread.

Starting in 2022, surveillance data indicates that men who have sex with men make up more than 80% of cases in the global clade IIb mpox outbreak *[27]*. Sexual transmission has also been documented in clade Ib *[16]*. Notwithstanding, anyone who has been in close personal contact with someone who has mpox is at risk of infection. Available data (prior to July 2024) suggest that among mpox cases with known status, about 50% are among people living with HIV. Data suggest that HIV per se is not a risk factor for mpox severity, however, people with severe immunosuppression, including uncontrolled HIV, are at increased risk of hospitalization and death due to mpox *[8, 28]* and are at risk for developing viral resistance to the frontline therapeutics *[29].*

2.4. Routes and timing of transmission

MPXV is transmitted through direct contact with infected persons exposed to infectious sores, scabs, or body fluids. Also, intimate contact between people during sex, kissing, cuddling, or touching parts of the body can result in viral transmission and disease spread.

There is evidence in clade IIb that some people shed and are infectious one to four days prior to symptom onset and that presymptomatic transmission may contribute to spread within outbreaks *[10, 11, 12, 13]*. In enzootic settings in Africa, infected animals can also transmit MPXV to individuals.

Additional less common routes of transmission are: contact with contaminated materials such as contaminated sheets, clothes, dishes or needles; and pregnancy, in which the pregnant woman may pass the virus to their foetus.

3. MPXV in wastewater and environmental waters

3.1. Potential inputs to wastewater and environmental waters

Human shedding

Shedding of MPXV may occur from multiple organs, including skin, respiratory secretions, saliva, urine, faeces, semen, blood and the anogenital tract of infected individuals *[30, 31, 32, 33]*. Most studies report use of molecular targets and are not able to differentiate infectious (replication competent) versus non-infectious virus. The magnitude of shedding is typically highest in the first two weeks after symptom onset with a progressive decline to below detection limits within four weeks for most individuals and by seven weeks for 90% of individuals *[32]*. Skin lesions have much higher viral loads compared to other sources *[32, 33]*. Further, individuals with immunosuppression, including those with advanced, uncontrolled HIV, are at higher risk of severe and prolonged mpox and a longer period of shedding *[8, 28]*. Given the multiple shedding sources, mpox viral material can be shed from infected individuals into both grey water through washing, bathing and laundry and black water through toilet use.

Most reported studies are from the multi-country outbreak of clade IIb since 2022. Shedding characteristics across all clades require additional research. The clade IIb mpox PHEIC outbreak was unusual in its mode of transmission and clinical presentation with a less extensive rash and location of lesions, so the more typical extensive mpox rash would logically be associated with higher viral load shedding into grey water through bathing and washing of clothes and bedding. Shedding with replication competent virus has been documented prior to and in the absence of symptoms in prospective cohort studies for clade IIb *[11, 12, 13]*.

Animal shedding

There are zoonotic reservoir hosts (clade Ia in Central Africa and clade II in West Africa) as well as potential expanded wild and domestic secondary or intermediate hosts including rodents *[4, 5, 6, 7, 34, 35]*. Several animal species have been identified as susceptible to MPXV, including rope squirrels, tree squirrels, Gambian pouched rats, dormice, non-human primates and other species. Faecal and urinary shedding has been documented in a number of primate and non-primate species in laboratory experiments *[30]*. However, uncertainty remains on the natural history of MPXV and further studies are needed to identify the animal reservoirs and how virus circulation is maintained in nature. There are also zoonotic infections for other orthopox viruses. The possibility of animal source contributing to WES OPX or MPXV detections should always be considered in choosing the appropriate diagnostic assay and in interpreting WES results with consideration of local context including presence of other endemic orthopoxviruses and epidemiology.

3.2. Target persistence and degradation

While DNA viruses are generally more stable as compared to RNA viruses, there is currently insufficient evidence to determine MPXV persistence and infectiveness in raw wastewater and environmental waters *[30]*. One study shows persistence of infectious MPXV in spiked untreated wastewater with slow degradation in the presence of chemical disinfectants *[36]*. While no cases of mpox have been reported to date from contact with contaminated wastewater or environmental waters, this data raises potential concern for environmental exposure to infectious mpox for humans and animals; for example to humans bathing in shared water as well as to susceptible species such as sewer-dwelling rodents. Further research is needed. Standard protections for infectious hazards including personal protective equipment are recommended for those with occupational exposure *[37]*.

To detect viral pathogen markers in wastewater and environmental waters, and interpret qualitative (detection above analytic threshold) or quantitative results of molecular targets, it is important to consider the sample type and starting concentration (viral loads entering the system), transit time and degradation rate to the sampling point as well as any persistence in biofilms. Temperature has been shown to be a key factor influencing the rate of DNA and RNA degradation with a positive non-linear and pathogen-specific relationship between wastewater temperature and degradation. MPXV-specific degradation rates and their determinants are a knowledge gap that requires additional research relevant to the interpretation of WES results, inclusive of the non-sewered settings with high ambient temperatures in tropical African settings of most interest.

3.3. MPXV WES experience

Since 2022 there has been wide at-scale national and subnational experience of WES for MPXV in parallel with case-based clinical Mpox surveillance in a wide variety of global settings (including in Europe, North and South America, Asia and Africa), with low and moderate mpox case incidence. These have been coupled with applied research to address key knowledge gaps, optimize methods and triangulate WES findings together with clinical results and other relevant data *[38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57]*.

Many WES MPXV programs, including those which have not (or not yet) resulted in publications, provide WES results for large geographic areas in public-facing dashboards to disseminate data to relevant parties and raise awareness of the location, trends and timing of mpox community circulation *[58]*. One well established public-facing, multi-pathogen example including MPXV is the United States Centers for Disease Control and Prevention's (CDC) National Wastewater Surveillance System (NWSS) *[23]*.

Results demonstrate that MPXV DNA is detectable in wastewater or wastewater derivatives (such as activated sludge from treatment plants) and correlates with reporting of clinical cases. These establish analytic feasibility for qualitative results with detection above assay limit of detection (all from low case settings with estimated high mpox case ascertainment) *[47,48, 49, 41, 54, 56]*. Studies in South East Asia including sewered as well as non-sewered settings have shown MPXV detections even in the absence of known cases in multiple countries where clinical reporting may be low due to multiple factors *[45, 46, 52]*. Various method optimization approaches have improved analytic sensitivity for WES detection *[37, 59, 60]*.

There is growing evidence to estimate the sensitivity of wastewater results to detect the presence of MPXV cases as well as positive and negative predictive values in sewered settings with relatively low case numbers *[48, 49, 60]*. An analysis of empirical data from the large national program in the United States of America estimated a weekly sensitivity of 32%, 49% and 77% for detecting respectively 1, 5 and 15 mpox cases in wastewater samples that represent thousands to millions of persons with high positive and negative predictive values of 62 and 80% respectively *[48]*. There is a trade-off between sensitivity and specificity and predictive values which can be purposively considered in light of surveillance objectives and public health utility *[54]*. WES results have been reported to provide early warning, suggest undiagnosed community transmission and/or precede case reporting allowing more timely public communications and other targeted responses *[59, 61]*.

There is limited published evidence showing wastewater quantitative MPXV (Clade IIb) correlating with clinical incidence *[58]*. However quantitative MPXV is not expected to be useful in settings with low prevalence (as has occurred with clade IIb post its peak in most global settings), with potential utility more likely in outbreak contexts with moderate to high prevalence such as those prevailing in the Democratic Republic of the Congo in 2024.

To date, most at scale MPXV WES experience and published studies have come from settings with extensive reticulated sewage systems from generally higher income country settings with MPXV Clade IIb during and after the PHEIC period *[61]*. Studies from Thailand and other south-east Asian countries provide additional evidence of MPXV detection in unsewered settings in the absence of reported cases *[48, 49]*.

Other WES programs and projects in unsewered settings provide additional evidence and technical and operational insights relevant to WES applications including the feasibility of identifying, sampling and interpreting results from diverse sampling sites and their associated populations; these include the long-established polio WES program, the multi-target WES program in South Africa (inclusive of MPXV), and the various pilot and research studies on typhoid and other pathogens in various countries in Africa, Asia and the Pacific Islands.

4. Overall mpox surveillance and response

4.1. Mpox surveillance and response objectives

There is a comprehensive global Strategic Framework for enhancing prevention and control of mpox including use of multi-modal collaborative surveillance *[62]*. Surveillance and response objectives are outlined in the March 2024 WHO Interim Guidance for surveillance, case investigation and contact tracing *[63]*. The primary goals of mpox surveillance, case investigation, and contact tracing are to detect new outbreaks, stop transmission, and contain ongoing outbreaks. This approach aims to protect people at risk in both endemic and new settings while working toward eliminating human-to-human transmission of mpox.

This requires a combination of early detection and effective responses in a wide variety of global settings, and with an understanding of risks associated with human-to-human transmission as well as any zoonotic circulation and human-animal interfaces.

In addition, due to viral evolution including mutations affecting some clade specific diagnostics, monitoring the genomic evolution of MPXV and its potential impact on performance of NAAT assays used is strongly recommended *[65]*.

Mpox surveillance and response approaches must be tailored to the specific local context. These contexts vary widely, spanning those newly affected as part of the clade IIb multi-country outbreak since 2022, as well as countries long affected by repeated clade Ia or IIa zoonotic outbreaks. They also include the unprecedented outbreak in the Democratic Republic of the Congo with ongoing human-to-human transmission via direct and sexual contact and emergence of clade Ib, and within country, regional and international spread of clade I. .

4.2. Existing surveillance systems/data sources

Case based surveillance with laboratory confirmation is ideal for national and international reporting using WHO standardized reporting forms *[65]*. Laboratory confirmation is important to confirm mpox and exclude other causes of febrile rash illnesses. However, in the Democratic Republic of the Congo and similar settings, most cases had been reported as suspected cases based on clinical assessment alone as laboratory confirmation had not been widely available prior to Aug 2024. Recent efforts have improved access to laboratory tests with an improvement of the proportion of clinical cases with laboratory confirmation from <10% to approaching 50% as of Aug 2024.

Since the PHEIC declaration, there has been an intensification of surveillance and response efforts at country, regional and global levels *[62, 67]*.

5. Mpox wastewater and environmental surveillance (WES)

5.1. Potential role of WES as part of multi-modal mpox surveillance

Multi-source surveillance systems using a “collaborative surveillance" approach *[67]* are those that provide relevant intelligence to make decisions that are fit for purpose in the country or geographic context at the lowest cost. Timely intelligence requires the availability, synthesis and interpretation of all relevant information and it is in this context that WES is discussed and not as a stand-alone source of information. Notwithstanding, for WES to be considered as part of the surveillance toolbox there must be specific and potentially impactful actions that are likely and made possible by WES results. A further ethical criterion is consideration of potential for harm including direct and indirect outcomes. As such WES for MPXV must identify specific actions and their likely outcomes within the overall local mpox surveillance system and response.

WES MPXV data (whether qualitative - above/below threshold, genomic and/or quantitative), where there is confidence in its interpretation, would be integrated with clinical and other information to:

- provide intelligence as to where mpox clades and subclades (including Ib) are circulating, as well as where they are not and inform public health decisions and actions in relation to population risk (in space and time) inclusive of targeting of communications, diagnostics, expanded surveillance, vaccines and other health system resources
- provide additional evidence and insights on evolving mpox epidemiological patterns including asymptomatic individuals, and/or infected individuals not being diagnosed and reported
- contribute to evaluation of the population-level effectiveness of case-based surveillance and other interventions

Presence of existing WES for other pathogens (e.g., polio, SARS-CoV-2 or other) provides opportunities to leverage any parts of the program and partnerships and decrease costs, to the extent that they are synergistic and also meet emergent mpox surveillance needs. In addition, the local sanitation system and practices are pertinent with wide global diversity. In most high and middle-income country settings, there is a high coverage of reticulated sewage systems for grey and black water. In contrast, many low-income countries have very low coverage, and grey water (from washing individuals and their clothes) frequently goes into surface water, while black water (toilets) predominantly involve closed septic systems which may also contaminate surface water drains and river systems. Given the high viral load in mpox skin lesions, grey water capturing bathing and showering rather than black water, may be of particular interest with implications for sampling choices. Choice of sampling location and interpretation of any sample result or results must consider the potential inputs from humans residing, working and/or visiting in or near the area as well as potential confounders.

Some countries already use WES for MPXV to complement case-based surveillance and strengthen progress toward the goals and objectives outlined above, however to date published reports are limited to clade IIb, mostly from higher-income settings with samples from sewered systems. Addition of WES as part of multi-modal mpox surveillance requires consideration of numerous factors.

These include:

a) the strengths and limitations of local mpox case-based surveillance considering availability of, access to, and uptake of, clinical and diagnostic services and the timeliness of reporting, as well as the role and extent of paucisymptomatic and presymptomatic MPXV transmission;
b) the relative general and specific advantages and limitations of WES; and
c) the specific epidemiologic and response context with specific actions associated with surveillance results.

General WES advantages include that it provides a geographic, population-based tool, with results that are agnostic to presence and timing of symptoms, health seeking behaviors and service availability, and that have favourable attributes for population coverage and cost. WES can be a very flexible tool with purposeful choices affecting and modifying sampling locations, type and frequency, analytic approaches and timeliness with turn-around time from sampling to results. There are also key limitations and knowledge gaps which are further described in sections E and G, particularly in relation to non-sewered settings and clade I subtypes relevant to the current PHEIC.

5.2. Specific surveillance objectives and associated use cases

Specific surveillance objectives and associated use cases for MPXV WES that may be relevant in the current PHEIC in specific contexts are:

1. Early detection, as well as reassurance of absence, of MPXV transmission

WES results and trends in strategic locations and complementing case-based surveillance may be applicable in routine ongoing WES surveillance or in a time-limited, agile surveillance initiated in response to a trigger as follows:

- **Routine surveillance – with ongoing monitoring at strategic sentinel locations** in settings with ongoing or intermittent community transmission, to inform the mpox response and evaluate its effectiveness. The mpox response includes timely communications to; raise awareness among health services and individuals at heightened risk, target resources including diagnostics and vaccines, and initiate further investigation and strengthen case-based surveillance if case and WES results are not concordant among other things. This is an established use case in sewered settings with MPXV clade IIb circulation as part of multi-target WES triangulating with case information. It provides, on occasion, early warning prior to, or in the absence of reported cases. Expanded applications for clade I, unsewered settings and areas with enzootic transmission require applied research.
- **Agile or enhanced surveillance - with initiation of time-limited monitoring at strategic locations** such as those near outbreaks and other areas at heightened risk, where there has been no recent cases, providing early detection which prompts geographically targeted actions, or reassurance of the lack of transmission with repeated negative results.*In the current PHEIC context, neighbouring or otherwise epidemiologically inter-connected countries/regions adjacent to a substantial mpox outbreak at heightened risk, such as countries in east and central Africa with clade Ib cases and other emerging hotspots with land and/or air travel links*
- **Detection and monitoring of spread of clade Ib and other clade I/II** with use of orthopox, mpox and mpox clade specific assays and/or amplicon sequencing (or other methods)

In the current PHEIC context, differentiation of subclades and identification of cocirculating clades and subclades from WES would be a cost-effective complement to the limited genomic information available from clinical specimens. This may include sentinel sites with high population coverage within countries (using samples from 1.1 or 1.2). Transport hub surveillance may also provide useful information about circulating strains. However, it requires publication of laboratory validated methods drawing on those used for clinical samples and noting rapid ongoing development and optimization.

2. Genomic characterization of the variable portion of MPXV

Should research demonstrate the validation and feasibility of amplicon sequencing or partial genomic sequencing of relevant regions from WES samples, an additional objective of genomic characterization may be added *[68]*. If successful genomic characterization could monitor genomic evolution with consideration of target regions for the current diagnostic NAATs and phenotypic shifts of concern as well as to potentially assess phylogenetic relatedness and linkages between human and animal MPXVs (integrated with human and animal clinical genomic surveillance and special studies). Relevant in settings with potential for genetic mutations and recombination (such as that prevailing on African continent in current PHEIC) as well as where case ascertainment or access to clinical specimens is limited and with consideration of relative costs and strengths of a population sample with high coverage as well as limitations of laboratory methods.

3. Timely identification of quantitative trends including increases, peaks and declines

There may be a role for quantitative trends in high-case load settings, as is used in SARS-CoV-2 WES. Subject to validation through further research, should quantitative trends be shown to be consistently correlated with case-loads with relevance to circulating subclade or subclades, there may be a role for quantitative trends in high-case load settings, as is used in SARS-CoV-2 WES.

Timely identification of quantitative trends including increases, peaks and declines with identification of hot spots of higher incidence which informs distribution of diagnostic and vaccine supplies and services, and evaluation of the effectiveness (or not) of the response. The level of granularity of sampling chosen is determined with consideration of the surveillance objectives, the limitations of case-based surveillance as well as feasibility and cost.

This is likely to only be applicable to sewered settings with higher case-loads (if they emerge) given unsewered settings have inherently higher variability and uncertainty. As part of the research any subclade specific differences in shedding and resultant WES quantification would also need to be assessed. Environmental surveillance using similar sampling and laboratory methods may also provide utility in One Health mpox surveillance to investigate and assess zoonotic reservoirs and secondary hosts and leverage any MPXV WES, however details are beyond the scope of this human-health focused summary.

5.3. Specific mpox response actions which may be informed by WES results

WES should always contribute to combined intelligence together with case and other information. Actions may include:

- Timely communications to communities at heightened risk to promote effective behaviour change including individual preventive behaviours (risk-reduction to exposure), mitigation

(isolating/risk-reduction if already exposed or unwell), health seeking actions (vaccine uptake, use of diagnostic tests, health services access and enabling timely contact tracing)

- Timely public health preparedness and response including geographically focussed testing, vaccinations and other population level interventions (noting importance of best use of scarce human and other resources)
- Clinical health system preparedness including promoting clinician awareness, diagnostic and clinical care logistics and human resource planning inclusive of sexual health services
- Additional agile WES (change of sampling location, frequency, TAT etc), case detection and other epidemiologic investigation to further characterize outbreak
- Timely identification of mutations relevant for guidance and use of diagnostic assays (if mutations affecting NAATs detected) and potentially further genomic characterization of clinical specimens
- Policy and operational response changes due to better understanding of epidemiology and public health threat and effectiveness of response

Discrepant positive WES results in the absence of clinical cases suggest a need to evaluate and strengthen access to and uptake of clinical and laboratory diagnosis and reporting. Further considerations in interpretation include prolonged shedding (e.g., from immunocompromised individual/s), zoonotic sources in enzootic settings, variability in disease expression by subtype (e.g., with presence of shedding from persons with asymptomatic or presymptomatic infection) and/or poor uptake of services related to marginalization or stigma.

5.4. Special use case for global sentinel surveillance at transport hubs

In general, advocates propose that a global WES system with twenty or more sentinel air transport hubs could provide additional intelligence, detect circulation of emerging pathogens and providing early warning and promote global equity *[70, 71, 72]*. Such transport node surveillance is not intended for case finding or contact tracing but rather timely information of global emergence and spread. To implement such a system requires collaboration and engagement of both supranational and national entities including the aviation sector and other WES partners. A proof of concept exercise was conducted in September 2024 to demonstrate synchronized sampling at major airhubs (airports and/or aircraft) with participation of more than 30 countries in all continents with testing of multiple pathogens including MPXV *[58]*.

In the context of the current mpox PHEIC, MPXV clades Ia or Ib detection in areas without previous cases would be of particular interest and require consideration from local and regional health authorities as to any additional WES or other surveillance, risk communication or other proportionate actions required. An MPXV (or other pathogen) detection in an airport or aircraft may be consistent with a transit traveller, viral persistence from an individual who exited the aircraft prior to arrival, or a person shedding after their infectious period and is therefore not synonymous with incursion of an infectious case. Reporting of mpox results to WHO is encouraged consistent with International Health Regulation obligations. Ethical and legal considerations including potential harm to individuals, businesses or countries as a result of mpox testing and detections must be considered in relation to appropriate sampling targets (including airports and/or aircrafts) as well as interpretation and reporting.

6. WES methodological considerations

6.1. Sampling

Overall, the sampling plan must correspond to and support the specific WES local surveillance objective/s with consideration of the local sanitation system (sewered and variety of unsewered settings) and mapping of strategic sampling points, and their associated catchments and relationship to populations of interest. There are technical and pragmatic considerations to balance including to what extent they leverage off, and are integrated with, existing WES programs (e.g., for polio, SARS-CoV-2 and/or other targets) and whether different approaches are required. Once sampling locations are identified, sampling implementation detail includes: sampling location access approvals, frequency of sampling, sampling type and duration, transport associated requirements and activities to achieve the desired end-to-end turn-around time. Triggers for agile surveillance (from clinical case information or WES MPXV single or repeated detections) and associated changes in sampling should also be identified a priori with consideration of possible scenarios.

For settings where there were no mpox cases before the outbreaks that resulted in PHEIC announcements (in 2022 and 2024), sample collection may be integrated together with existing and ongoing WES programs (i.e., including MPXV together with other pathogens as part of any local existing WES program). Some increase in sampling locations or frequency may be initiated in places and during periods of heightened risk (eg for clade 1a and Ib to characterize spread to nearby countries or sub-nationally in countries with known incursions or outbreaks, while for clade IIb strategic locations where risk of amplification is high may be large international pride festivals or similar). Given the patterns of spread, it is important to engage key partners in codesign of sampling strategies and other key aspects of the program; considering predominant spread through networks of men who have sex with men globally for clade IIb, sexual transmission involving commercial sex workers, long distance truck drivers, as well as men who have sex with men for clade Ib, children affected by household level transmission for clade I, as well as human-animal interactions associated with epizoonotic transmission.

In most African settings affected by recurrent epizoonotic outbreaks and the recent spread of subclades Ia and Ib, there are predominantly unsewered systems and, often, an established polio environmental surveillance program with monthly sampling from well-defined strategic sampling locations and established sampling transport to within country and regional laboratories. Sampling plans should consider where there might be alignment with existing polio environmental sampling to meet mpox objectives, these locations are shown in the comprehensive polio surveillance dashboards *[73]*. Care must be taken to ensure any such integration strengthens and does not weaken polio surveillance and eradication efforts. While there may not be local laboratory capability, agile surveillance approaches may still be considered provided sample collection and sample transport to a regional laboratory with MPXV WES capability is feasible. Agile surveillance may be triggered due to an increased local risk (e.g., due to local case detection, outbreaks or heightened risk from hotspots with travel links).

However, key knowledge gaps remain about the optimal WES sampling methods for MPXV especially in diverse unsewered settings. These require additional research before at scale use can be recommended. Various sampling types have been used successfully for MPXV detection (including

grab, composite, passive sampling with electronegative filters, wastewater sludge and settled solids), however there have been no comparative studies reported to date. For other pathogens such as SARS-CoV-2, grab samples have been shown to be less sensitive compared to other methods (consistent with their inferior temporal coverage), however grab samples are the established and widely-used method of pragmatic choice for the polio program. Sampling of settled solids in wastewater treatment plants results in higher MPXV positivity rates compared to samples from suspended solids or liquids *[46]*. Given persistence of DNA viruses including MPXV in the environment, these results may reflect MPXV prevalence over different time periods with extended cumulative prevalence versus a measure closer to point prevalence. Most surveillance objectives seek to identify recent infections with incidence or point prevalence desirable, such considerations inform pragmatic sampling and analytic choices.

6.2. Laboratory

WHO has provided updated interim guidance for diagnostic testing for MPXV *[19]* as well as target product profiles for tests used for MPXV diagnosis *[74]* with ongoing technological advances including in point of care and near point of care diagnostics. The analytic methods used in WES are similar to those for clinical diagnostics, but given the complex population pooled matrix, additional steps are required for processing and analysis. For WES widely used and validated molecular methods, including single-plex and multiplex methods are able to detect the presence of MPXV DNA above detection limits, including with faecal biomarkers (such as pepper mild mottle virus, enteroviruses or other biomarkers) as a quality control to confirm presence of human faecal materials *[58, 75]*.

There are a variety of methods which have been used to successfully detect MPXV and OPXV in wastewater and environmental samples however as an emerging field there is no standardized recommended method established *[44]*. In low case settings, methods to optimize system sensitivity and reduce the occurrence of false positive results with confirmatory methods and quality assurance is paramount. These include purposeful sampling as well as laboratory considerations which may require deliberate trade-offs such as those between sensitivity and cost-efficiency *[54]*. Generally, genomic characterization requires higher concentrations in environmental samples than PCR, so PCR is more sensitive than sequencing in low case settings. Targeted PCR can be used to detect generic OPXV or MPXV as well as clade or subclade specific PCR (including for clade Ib),It is important to use test that target viral conserved genes *[19]*. Subclade differentiation and identification of co-circulating subclades through targeted PCR probes with confirmation by amplicon sequencing and/or other methods are a priority for WES validation, given the coverage, cost and timeliness advantages a population level sample can provide over clinical samples alone. This would parallel the WES use case for SARS-CoV-2 with genomic characterization of variant incursion and spread with relative variant abundance. Confidence was established in SARS-CoV-2 results by evaluation which showed high correlation between WES and individual clinical genomic surveillance results as well as independent confirmation at low case-loads to exclude false positives.

Evidence is emerging of varied WES genomic analytic applications. Preamplification has been shown in one US study to significantly increase sensitivity of testing in a low case setting, reduce false positive results and result in a high success rate for confirmatory Sanger sequencing *[59]*. While enrichment approaches were used in another study from Brazil with long read sequencing *[50]*. Long

read sequencing of clinical cases have resulted in identification of novel mutations in both Clade I and II *[17, 76, 77]*. Novel applications for WES are being developed and researched with targeted short read amplicon or partial genome sequencing of variable regions or other regions of interest such as those related to diagnostic targets, similar to those developed and used for SARS-CoV-2 *[69]*. These have potential advantages in terms of population coverage and cost in relation to clinical case-based sequencing alone. Further studies are needed in high-case load settings to evaluate correlation of WES quantitative trends against case and hospitalisation trends to determine WES utility to track dynamic trends; a single study with Clade IIb in low case, sewered settings in the US provides preliminary evidence to support proof of concept similar to proven WES applications for SARS-CoV-2 and other viral pathogens *[58]*. Further, given the centrality of NAAT diagnostic testing and the presence of mutations which affect diagnostic sensitivity for MPXV WES may also have a role in monitoring for emergence of mutations at sites of importance for diagnostic tests *[19, 25, 78].* Genomic reference sequences are available, CDC provides one such published sequencing reference and related information for laboratory personnel *[23]*. A novel PCR for clade Ib is also described *[18, 19]*.

There are local and international requirements related to MPXV and subclade specific identification, sample handling, storage and transport of samples and viral isolates *[19]*. These vary by country; for example, inthe USA, Clade I is a select agent and Clade I MPXV (non-culture diagnostic specimens) are now classified as Category B infectious substances *[14]*. Further, local circulation of zoonotic orthopoxviruses (OPXV) must be considered in order to choose and interpret results of generic OPXV assays, for example, there are North American poxviruses (volepox, skunk pox and racoon pox) and tests in that region must be evaluated for their sensitivity and specificity *[79]*. Each locale would need to consider any endemic zoonotic OPXVs as well as enzoonotic and emerging reservoirs of MPXV in the choice of methods and interpretation of results.

6.3. Reporting and communications

Globally, there are a growing variety of country and location specific dashboards including WES results and useful visualisations for MPXV as well as other pathogens (e.g., SARS-CoV-2, influenza, norovirus) with quality control or normalization typically using faecal biomarkers (CDC, 2024). While there is some convergence and increasing alignment on best practice content which provides integrated views of case based, WES and other surveillance results, there does remain a wide diversity of analytic and reporting approaches. Best practice approaches require modification of existing reporting platforms, or development of new platforms, specifically that allow integration, visualisation and use of multiple data sources including clinical, WES and any other relevant data (e.g., zoonotic data). Interactive interfaces that meet varied user needs including those of local decision makers and key stakeholders engaged in the overall mpox response would be desirable. If public facing, key target audience and stakeholders include clinicians and individuals at risk requiring sensitivity to user needs to avoid misunderstanding and prevent stigma and other harms. The global polio dashboard provides such a best practice example with a disease specific integrated model with access to case, environmental surveillance and other data and which includes quality control metrics and is tailored to multiple user needs at country, subnational and supranational levels.

Genomic data represents a specialized area where there is a need to consider how to integrate, visualize and access WES derived location and pooled population information alongside individual

clinical sample information. This has been a long-standing issue which has not yet been resolved for other pathogens such as SARS-CoV-2 undermining the utility and timeliness of insights from WES.

6.4. Ethical and legal considerations

There are critical ethical issues to consider for any surveillance, including WES, which may differ according to context, including for the protection of population groups who may be at risk of stigma in difficult social or legal contexts, such as men who have sex with men, or sex workers in many countries, and other vulnerable or marginalized groups most affected by mpox.

Acceptability, social licence, ethical and legal issues are relevant for any application of WES. However, mpox raises specific concerns. For mpox there are critical ethical issues which require consideration for any surveillance activity, inclusive of WES, given sexual transmission of MPXV, with the predominant Clade IIb transmission through networks of men who have sex with men and demonstration of Clade I male to male and heterosexual transmission, coupled with the need for the protection of population groups who may be at risk of stigma in difficult social or legal contexts *[80]*. Ethical considerations also encompass optimizing program effectiveness; which requires relevant information to reach and be understood by the individuals at highest risk and this information not to be misused for punitive or stigmatizing non-health goals. The potential for harm and approaches to mitigating harm must be considered in program design from sampling location though to data use and reporting. Both require engagement and codesign of the WES system with key partners including those at highest risk. This is especially relevant for any use of more localized surveillance where the risks of stigmatization and discrimination are heightened but where there also may be greater potential benefits.

7. Considerations for integrated surveillance and multi-target WES

7.1. Integration of MPXV WES into existing mpox surveillance and response

WES is acknowledged within the 2024-2027 Strategic Framework and the WHO March 2024 WHO global guidance for MPXV surveillance noting its potential and limitations including the relative lack of information for Clade I and non-sewered settings. The declaration of the PHEIC and the need for timely results to inform the response, highlights the urgency to address WES knowledge gaps. It will be helpful to learn from country level experiences to optimize integration; including real-time solutions to better visualize and use combined data to produce mpox intelligence which inform public health actions. There are numerous national and subnational examples of use of WES for MPXV in parallel with case-based clinical mpox surveillance, particularly during the PHEIC period. Some countries (e.g., China and the USA) have ongoing integrated MPXV surveillance in 2024. Table 2 provides an overview of MPXV related clinical and WES surveillance at the national level in the USA noting these provide public-facing information.

Table 2: Surveillance systems which include MPXV/Mpox in the USA

Surveillance System/Data Source	Outcome(s)	System Type	Frequency	Geographic Resolution
National Electronic Disease Surveillance System Base System (NBS) (www.cdc.gov/nbs/mpox-response.html)	Illnesses; Epidemiologic, laboratory, and clinical data	Integrated information system	Rolling	Jurisdiction-level
National Notifiable Diseases Surveillance System (NNDSS) (www.cdc.gov/nndss/index.html)	Infections and non-infectious diseases and conditions	Voluntary reporting of nationally notifiable diseases	Weekly and annual	Jurisdiction-level
National Wastewater Surveillance System (NWSS) U.S. Mpox Wastewater Data | National Wastewater Surveillance System | CDC	Mpox virus detection in wastewater in the past 4 weeks	Integrated information system	Rolling - biweekly or weekly	Sewage catchment (mapped to state)

7.2. Integration of MPXV as part of multi-target WES surveillance

Presence of an existing WES program (e.g., polio, SARS-CoV-2, other) provides opportunities for cost-effective program synergies to add additional targets in routine or agile surveillance, if and when sampling, analytic and reporting processes align and drawing on existing specialized human resource capacity and capability. This also requires consideration of how both programs may be strengthened as well as mitigation of any potential harms so that existing WES (such as that for polio eradication) are not weakened and WES for MPXV objectives are achieved. MPXV may require special sampling considerations, such as:

- Given the predominant MPXV shedding from skin and mucosal lesions and typical focus on faecally contaminated waters, knowledge of behavioural practices and grey water sources is needed to ensure sampling locations capture bathing as well as washing of clothes.
- For key and/or vulnerable populations and associated sentinel sites,(e.g., children, men who have sex with men, sex workers and their clients, prisoners, refugee and displaced persons in camps among others) with consideration of geography, mobility and changing epidemiology.

Optimization of sampling, pre-analytic and analysis for MPXV is also needed with some studies suggesting a need for differentiated procedures for the enveloped double-stranded DNA MPXV as compared to enveloped RNA viruses such as polio, SARS-CoV-2 and influenza.

8. Knowledge gaps and research priorities

8.1. Key limitations

Key strengths of WES described include attributes drawing on place and time-based pooled population samples which are highly flexible, relatively low cost, and agnostic to symptoms or access to services, coupled with a range of sensitive molecular techniques and bioanalytical approaches. Limitations specific to MPXV include:

- Paucity of information of WES use for subclades Ia and Ib and in unsewered settings
- Choice and characterization of sentinel sites relevant to strategic areas and populations of interest (including in a variety of unsewered settings)
- Scalable analytic methods including subclade-specific PCR assays and amplicon sequencing for confirmation as well as genomic characterization
- Early stage of development, harmonization and standardization of WES for MPXV affecting all aspects; including optimal sampling, wet and dry analytic methods, bioinformatic pipelines, interpretation, integration and reporting
- Relative lack of integrated WES and case-based mpox disease surveillance and response to date (inclusive of data pipelines, visualisation and communications)
- Limited evidence of correlates of clinical data and quantitative WSE levels
- Limited evidence of degradation and stability in wastewater for MPXV

8.2. Research priorities

There are several applied research priorities based on the potential for improved MPXV surveillance in the context of the current PHEIC. Recommended areas of applied research include:

- Context-specific value addition of routine and agile WES for MPXV to current mpox surveillance and response considering all subclades in varied global settings, but especially clade Ib
- Sensitivity of WES PCR detection for early detection with low case loads and rapid confirmation methods (and which may include presymptomatic and paucisymptomatic shedding)
- Feasibility of specific public health applications with WES implementation in contexts not well studied such as unsewered setting, particularly in enzootic settings in Africa
- Interpretation of WES results with consideration of human and zoonotic sources
- Resource requirements for initiation and maintenance of routine and agile WES for MPXV
- All of above - with a priority for the clade Ia and Ib spread in Africa but also with consideration of applicability to all subclades and any emerging sub-lineages or recombinants and diverse settings
- Harmonization and standardization (where feasible and appropriate) of WES methods across the work flow and establishment of best practice approaches and requirements
- Method validation for quantitative results with assessment of clinical correlates and/or public health significance to establish confidence in these metrics

The existence of MPXV specific knowledge gaps does not preclude its utility and application in the PHEIC, noting that early detection is a compelling use case where case-based surveillance is not strong or timely. As shown in the COVID pandemic, rapid applied learning can be accelerated to fill knowledge gaps and expand the use cases.

9. Methods

A WHO steering group and WES expert review group, each listed in acknowledgements was convened for a WHO WES package called *"Guidance on prioritization and implementation of multi-pathogen wastewater and environmental surveillance (WES) to support public health decision-making"* (in press) which includes a pathogen specific summary for MPXV. Following the announcement of the PHEIC for Mpox in Aug 2024, and working with the steering group and expert review group for the wider WES package the target sheet for MPXV was expedited as a standalone considerations document.

Source of evidence

Multiple lines of evidence were used to inform this document. The principal source was articles from published peer reviewed journals in English since 2000 derived from a search of with terms 'wastewater' or 'sewage' and 'monkeypox', "MPXV" or 'mpox' considering all study designs. A total of 36 studies of WES for MPXV application to inform potential use cases (Section) and 19 studies to inform methodological consideration (Section 6 and 7).

In addition, evidence from public-facing national WES dashboards and curated websites were drawn upon, including; WHO and CDC published documents on mpox, Global Consortium for Wastewater and Environmental Surveillance (GLOWACON) – inclusive of the Encyclopaedia Cloacae, and pathogen specific e-resource for mpox and US-CDC– National Wastewater Surveillance System (NWSS).

Evidence synthesis and review process

Evidence was synthesized by lead consultants following the document structure agreed with the steering group. Draft evidence review was then reviewed by WHO and US CDC steering group and mpox WHO and CDC disease experts. The draft was then reviewed by mpox sub-group members of the expert review group. Their input was consolidated in a group meeting to reach consensus. A final document was then shared for error checking and no objection.

Plans for updates

WHO continues to monitor emerging evidence and will issue a further update if substantive findings require this. Otherwise, this document will expire six months after the date of publication.

Selection

Lead consultants and external expert reviewers were selected via research and practitioner networks working on WES globally. Selection aimed for a balance of research and implementation experience, gender and regional representation.

Declaration of interests

All members of the group completed declarations of interest, which were reviewed in accordance with WHO principles and policies and assessed for any conflicts of interest. No conflicts of interest were identified that required individuals to abstain from consensus decision making.

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WHO Steering Group

Lead consultants

Monica Nolan, WHO consultant medical epidemiologist, Australia; Daniel Deere, WHO consultant environmental microbiologist, Australia.

Expert contributors

Juliana Calabria de Araujo, Universidade Federal de Minas Gerais, Brazil; Megan Bias, National Wastewater Surveillance System, United States of America (USA); David Berendes - Global WASH United States Centers for Disease Control and Prevention (CDC), USA; Megan Gerdes, National Wastewater Surveillance System, USA; Nalia Ismail, Instituto Nacional De Saude, Mozambique; Tim Julian, Swiss Federal Institute of Aquatic Science and Technology (eawag), Switzerland; Amy Kirby, US Centers for Disease Control and Prevention, USA; Alex Kossick - Global WASH, CDC, USA Supriya Kumar (Observer), Bill and Melinda Gates Foundation, USA; Placide Mbala, Institut National d'Hygiène Publique, Democratic Republic of the Congo; Kerrigan McCarthy, National Institute for Communicable Diseases, South Africa; Gertjan Medema, KWR Water Research Institute, The Netherlands; Scott Meschke, University of Washington, USA; Jennifer Murphy, Global WASH, CDC, USA; Michael Owusu, Kwame Nkrumah University of Science and Technology, Ghana; Samuel O. Oyola, International Livestock Research Institute, Kenya; Tarja Pitkänen, Helsinki One Health, Finland; Maya Ramaswamy, Global WASH, CDC, USA; Heather Reese, National Wastewater Surveillance System, USA; Renee Street, South African Medical Research Council, South Africa; Mukhlid Yousef, National Institute for Communicable Diseases, South Africa.

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