Wastewater and Environmental Surveillance Summary for Poliovirus

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This document provides information on wastewater and environmental surveillance (WES) for poliovirus. It should be used together with the accompanying *WES Guidance for one or more pathogens*, which includes general and cross-cutting information (available here). Except where cited otherwise, information has been drawn from existing World Health Organization (WHO), United States Centers for Disease Control and Prevention (US-CDC) and the Global Polio Eradication Initiative (GPEI) publicly-available sources, current at the time of writing.

WES for Poliovirus at a glance

- Polio is a target for eradication of high global public health significance.
- WES for polioviruses is actionable, technically and operationally feasible, and acceptable in varied sanitation settings. It is well integrated as part of the global polio eradication program.
- However, poliovirus WES remains predominantly a single pathogen program. Integration of other targets within one or more WES workflows is promising but is at an early phase.

Table 1 : At a glance assessment of key WES criteria for poliovirus (sewered and non-sewered)[a,b]

Setting	Categorical Assessment (CA) / Strength of Evidence (SoE)	Public Health Significance	Actionability / Relative value	Technical Feasibility	Operational Feasibility	Acceptability	Optimisation: Integrated disease response	Optimisation: Multitarget WES
Sewered	CA							
	SoE							
Non-sewered	CA	not separated by sewered category						
	SoE							

Key:

1. Categorical Assessment (CA) of criteria

Category	Code	Description
High		Criteria is evaluated as met at the highest level
Intermediate		Criteria is evaluated as met at an intermediate level (it may be that not all sub-components of the criteria are met)
Low		Criteria is evaluated as low
Not-supported		Criteria is evaluated as not supported
Not applicable		Criteria is not applicable OR cannot assessed due to inadequate evidence

2. Strength of evidence (SOE)

Evidence level	Code	Description
Strong		High quality consistent evidence, including from multiple relevant studies/settings, at scale, over a prolonged period, with evidence from program settings, not only from research studies or short projects.
Moderate		Relevant evidence is available but does not meet criteria for 'Strong' classification.[c]
Inadequate evidence		Evidence is inadequate and further study/evaluation is needed

[a] Further description of the criteria used to assess the applicability of WES for a specific pathogen, as well as the methods used to evaluate them, is included in *WES Guidance for one or more pathogens*. The assessment in Table 1 provides a snapshot at the global level, but country level assessment may differ.

[b] Sewered settings refers to closed reticulated sewage systems. Non-sewered settings refers to the diverse settings which are not 'sewered', including open drains and community sampling points. Individual small septic tanks at residential or building level are not viable to sample individually and are not considered here separately. Most WES evidence to date is reported from reticulated sewered settings, often from high-income settings. Yet much of the global population is on heterogenous non-sewered systems and this has implications for assessment of various WES categories.

[c] Evidence classified as 'Moderate' meets one or more of the following criteria: not from numerous settings, for a short period, without program-level evidence, and/or where findings are not consistent or of high quality.

Summary

- Polio remains a **Public Health Emergency of International Concern (PHEI)** with an ongoing **global eradication** campaign through the GPEI.
- **Poliovirus includes** wild poliovirus (WPV) **type 1** (WPV1) and circulating **vaccine-derived polioviruses** (cVDPV) for the three serotypes which are causing paralysis and other severe illness in humans. **WPV types 2 and 3 have been globally eradicated**. There are no zoonotic hosts.
- Surveillance **approaches differ by setting**, where poliovirus: a) is endemic, b) has a heightened risk, or c) has lower risk of reintroduction, d) outbreak exists, and e) in future – post eradication.
- Poliovirus surveillance includes both event-based surveillance of acute flaccid paralysis (AFP), and WES. Positive results from either are **actionable** by health authorities.
- **Routine WES** at sentinel sites is used to detect poliovirus as well as reassure of its absence. **Agile WES** (with additional locations and more frequent sampling) may be triggered by routine WES detections, case detection or a containment breach. It is used to characterize the extent of community circulation, inform the response and assess its effectiveness.
- The **technical feasibility** of WES for poliovirus is well established for detection and genetic characterization, which allows differentiation of polioviruses and phylogenetic comparison to clinical isolates. However, there are known limitations that may impact the effectiveness of the whole WES process, from sampling to genetic characterization of isolates; periodic grab sampling, transportation to laboratory, and characterization of complex virus mixtures can delay results and/or limit sensitivity. Direct detection using molecular methods and evaluation of alternative WES sampling methods is increasingly used.
- **Operational feasibility** of poliovirus WES has been demonstrated. There is considerable at-scale experience of sampling in sewered systems as well as non-sewered sanitation systems[1]. Sample handling, analysis and reporting are standardized with broad adherence to biosafety and biosecurity protocols through the Global Polio Laboratory Network (GPLN).
- **Integrated surveillance opportunities**:
    - WES is well integrated in all key aspects of the polio surveillance and response program. Poliovirus offers a model for other diseases on integrating WES as part of multimodal surveillance.
    - Poliovirus WES programs may be leveraged to include other pathogens at low marginal costs (where workflows align), with potential to enhance equitable, cost-effective surveillance and strengthen epidemic/pandemic preparedness and response capability.
- **Poliovirus WES** effectiveness may be improved through **applied research** in the following areas (consistent with the GPEI Strategy):
    - Improvement of the **sensitivity and timeliness of WES methods** which are feasible in relevant and varied global settings.
    - Optimization of **cost-effective, safe and sustainable poliovirus WES** through the polio eradication and post-certification phases, including biosafety considerations
    - Evaluation and optimization of the **inclusion of other targets in multitarget WES** while maintaining or strengthening core poliovirus WES effectiveness.

[1] *Referred to as structured and non-structured sewage systems in the Global Polio Eradication Programme.*

Contents

1. General information

1.1. The virus, associated disease and risk factors

Poliovirus is the causative agent of polio (also known as poliomyelitis). It is a small non-enveloped RNA virus within the genus enterovirus. WPV1 remains in circulation, while WPV types 2 and 3 have been eradicated and are retained only in secure laboratory repositories. Rarely, there are also mutations or re-combinations in the live attenuated oral polio vaccine strains which revert to become vaccine-derived polioviruses (VDPV), that can be transmitted from person to person and then categorized as circulating vaccine-derived polioviruses (cVDPV) . While cVDPV can arise with all three serotypes, serotype 2 (cVDPV2) is the predominant cause of outbreaks. These are transmissible to other persons and cause outbreaks and disease. Clinically, WPV and cVDPV are indistinguishable. Poliovirus causes no symptoms in most of those infected, and relatively mild flu-like symptoms in approximately 25% of those with acute infections. However, more serious symptoms, such as meningitis and paralysis, occur in approximately 1-5% and 0.05-0.5% of infected persons, respectively. Children are disproportionately affected. Other, non-polio enteroviruses such as serotype D68 can cause polio-like disease with acute flaccid myelitis.

1.2. Global burden and geographic distribution

Polio (both WPV and cVDPV) remains classified as a PHEIC, because poliovirus is highly infectious and without ongoing efforts it would again become a pandemic. When the GPEI began in 1988, poliomyelitis paralyzed hundreds of thousands of children every year. Since its inception, the number of WPV cases has reduced by > 99.99%. This has been achieved through a combination of community mobilization, routine and supplementary vaccination, improved water, sanitation and hygiene (WASH) practices and enhanced surveillance and outbreak responses.

Global eradication is at the end stage. WPV serotypes 2 and 3 were declared eradicated in 2015 and 2019 respectively. Five of the six WHO regions have been certified free of indigenous WPV, with the WHO African Region certified in August 2020. WPV1 remains endemic only in Pakistan and Afghanistan.

Since 2012, paralytic poliomyelitis cases due to cVDPV have surpassed those due to WPV. cVDPV outbreaks are concentrated in lower-income countries and conflict-affected areas where the Oral Polio Vaccine (OPV) is still in use and vaccination rates and sanitation systems are sub-optimal. The GPEI is implementing a long-term strategy to decrease the incidence of cVDPV by phasing out the use of Sabin OPVs in favor of inactivated polio vaccines (IPV) as well as genetically modified, more stable OPV versions (e.g., Novel oral poliovirus vaccine type 2, nOPV2). However, the transition has been slow and complex, especially in lower income countries.

1.3. Routes of transmission

Poliovirus is transmitted from person-to-person, mainly through the fecal-oral route or, less frequently, by a common vehicle (e.g. contaminated water or food). There are no zoonotic reservoirs with amplification outside human hosts.

1.4. Eradication and post-certification era

Following successful eradication of WPV1 and cVDPV there will be a shift in surveillance priorities. In this period, the primary risks of a potential resurgence of polio would be related to the continued use of OPV and emergence of cVDPV including that associated with chronic excretion of VDPVs by persons with immunodeficiency (iVDPVs)[1]. Accidental release of poliovirus from a laboratory or vaccine manufacturing facility and malicious release due to bioterrorism represent further risks[2–4].

2. Poliovirus and wastewater and environmental waters

2.1. Potential inputs to wastewater and environmental waters

Human shedding: Poliovirus may be shed by an infected person as well as by individuals who have received the OPV vaccination.

Poliovirus replicates in and is shed from the nose and throat for a few days and from the gut for several weeks, with high levels in the feces[5]. The frequency, pattern and amount of viral shedding varies widely between individuals and may be intermittent. Fecal shedding in immunocompetent unvaccinated individuals typically is high in the first two weeks, with a peak around 6-8 days post exposure. Shedding is usually cleared by 4-6 weeks with an average peak level shedding of 10e5 viral particles per gram of stool[6–8]. Persons with primary immunodeficiency may excrete high levels of poliovirus over a very prolonged period[9].

As a live attenuated virus, OPV also replicates and is shed in feces after vaccination. IPV is an inactivated virus vaccine and does not replicate or result in viral shedding. Reference to local polio vaccination schedules is required to determine potential local OPV-derived inputs. Detection of OPV and OPV-related virus in the wastewater is expected in areas with routine OPV vaccination. Higher levels of these are associated with supplementary OPV vaccination campaigns.

Other potential polio-related inputs into wastewater include accidental release of poliovirus from a laboratory or vaccine-manufacturing facility or malicious bioterrorist release.

Zoonotic shedding: There are no zoonotic (or non-human) inputs.

2.2. Target persistence, degradation and risk of infectious virus

Poliovirus degradation and persistence in the environment is well characterized; there is little degradation under the conditions and transit time periods associated with diverse sanitation systems across different climate zones. This, combined with the elevated levels of poliovirus fecal shedding and available analytic methods, have made polio an ideal target for wastewater and environmental surveillance.

Once excreted, poliovirus can survive for weeks at room temperature, including in aqueous media, because (as a non-enveloped virus) it is relatively stable to UV light and humidity. Lower temperatures and the presence of organic matter (as found in wastewater) slow down degradation rates, while increased temperature is associated with more rapid decay[10]. There is evidence that poliovirus partitions with the solid component of wastewater, with higher concentrations found in sludge fractions[11].

Exposure to poliovirus and other infectious pathogens might pose a risk to sanitary workers and others in contact with wastewater and human contaminated environmental waters. Standard protections for infectious hazards are recommended for those with occupational exposure.

3. Poliovirus surveillance

3.1. Overall poliovirus surveillance and response

The GPEI eradication strategy 2022 – 2026 (and extended to 2029) defines the strategy to achieve global polio eradication[12]. The goals are to:

- Permanently interrupt all poliovirus transmission in endemic countries.
- Stop cVDPV transmission and prevent outbreaks in non-endemic countries.

The surveillance-related strategic objective is to enhance detection and response through sensitive surveillance that provides the program with critical and timely information for action. There are two main types of surveillance to detect poliovirus. Clinical AFP case-based surveillance and WES. In addition, there is surveillance for poliovirus among individuals with primary immunodeficiency disorders (PIDs), referred to as immunodeficiency-associated vaccine-derived poliovirus (iVDPV) surveillance. This is done through broad enterovirus surveillance systems and special supplementary studies.

AFP surveillance and WES are used together for integrated polio surveillance. Both are supported by the GPLN and the polio data and information management system.

AFP surveillance is the primary surveillance approach. It includes finding and reporting children with AFP, transporting stool samples for analysis, isolating and identifying poliovirus in a GPLN- accredited laboratory and mapping the virus genetically and geospatially to determine its origin. There is active AFP surveillance in endemic, outbreak and high-risk countries and passive surveillance elsewhere. Nevertheless, case-based surveillance is typically a lagging indicator of poliovirus circulation given the low percentage of poliovirus infections that result in paralysis as well as the multiple weeks from symptom onset to laboratory confirmation.

WES supplements AFP surveillance and has the following benefits:

- WES can detect silent poliovirus circulation, capturing asymptomatic or pauci-symptomatic infections in the community, especially in settings with high humoral immunity and low-mucosal immunity (e.g. countries using only IPV (and no OPV) in routine immunization).
- WES population coverage and frequency can be designed cost-efficiently to meet polio surveillance needs - balancing timeliness of results and population coverage against program cost.
- Like clinical specimens, WES samples can also be used to identify the type of poliovirus, whether oral vaccine strains (reflecting routine or supplemental OPV delivery), WPV, VDPV; serotype; genotypes, and phylogenetic mapping can also be used to determine origin.
- In addition to routine surveillance, WES can be used in an agile response to an outbreak (with AFP or WES identification of WPV or cVDPV) with additional sampling locations or frequency to characterize the extent (or not) of circulating poliovirus.

Biosafety and biosecurity are high priority for the program, given the risk associated with accidental or malicious release of poliovirus (including viral isolates), as well as concerns for the occupational health and safety of staff. There are standard protocols for the safe handling and containment of specimens.

3.2. Poliovirus WES experience

There is extensive and longstanding experience of using poliovirus WES together with AFP surveillance from the dynamic phases of the polio response through to current end-game eradication efforts in more than 80 countries. In certain areas and contexts, WES has been shown to significantly increase the sensitivity of surveillance for poliovirus compared to AFP surveillance alone.

As early as the 1940s it was known that poliovirus was detectable in sewage [13]. Use of WES for poliovirus has been integrated as part of the GPEI for over 20 years. In 2003, WHO released Guidelines for Environmental Surveillance of Poliovirus Circulation [14]. In 2015, the GPEI released updated guidance as well as an acceleration plan to expand environmental surveillance, prioritizing countries with endemic or recent endemic disease, current or recent cVDPV outbreaks and ongoing use of OPV [15]. In 2023, the GPEI published Field Guidance for the implementation of environmental surveillance for poliovirus with a focus on programmatic and operational aspects such as site selection, sample collection and transport and the use of data for action [16]. These are complemented by other GPEI guidance[17] and supporting materials and tools for use by national polio surveillance programs and partners.

Together, these documents guide and support the established standardized practices of polio WES as part of the GPEI. Common standardized features of these WES polio program activities include:

- Sentinel site selection – considering key factors such as coverage of populations at highest risk, presence of convergent sewage systems and feasibility of sampling.
- Periodic sampling – typically using grab samples and monthly frequency.
- Sample collection, storage and transport – supervision and procedures which promote sample quality, the safety of personnel and documentation with chain of custody.
- Establishment and maintenance of an accredited GPLN implementing standardized WES methods – typically using two-phase sample concentration, viral isolation on cell culture, followed by genetic sequence confirmation to differentiate WPV, cVDPV and Sabin-like virus and phylogenetic mapping against all known poliovirus sequences from clinical AFP case and environmental samples.
- Information management tools – to enable timely visualization of AFP case and WES data to support decision making.
- Actionable plans in the case of single and repeated positive WES results – including for expanded WES, strengthened AFP case surveillance and other specific contextualized actions.
- Continuous quality improvement systems with monitoring of key metrics – which enable corrective actions including the strengthening or discontinuation of poorly performing sampling sites.

Surveillance objectives and associated WES approaches differ by setting and over time as the program progresses towards eradication.

Evidence that well-implemented WES significantly increases the sensitivity of surveillance for poliovirus in certain areas provided impetus for accelerated expansion from 2013 onwards [18]. WES program experiences demonstrate its value to provide early detection ahead of, or in the absence of, AFP cases, as well as to characterize the extent of an outbreak and its successful control. Phylogenetic analysis of WES and case genetic data has shed light on the source and transmission chains of poliovirus outbreaks. Repeated absence of detection has also provided useful context-specific information especially following prior endemicity or outbreaks.

There is extensive documentation of the specific value of polio as a powerful supplementary, sentinel-based detection system[14,18,12,16,15,20,21]. WES in different epidemiologic contexts and settings. **Illustrative examples** include:

- **Settings with endemic WPV:**
  - In Pakistan and Afghanistan, extensive WES has improved sensitivity and early detection over AFP alone and showed ongoing WPV1 circulation and geographic spread despite a reduction in reported AFP cases[22–24].
  - Historically, in Egypt and India WES was used to triangulate and corroborate the declining AFP case trends prior to successful elimination[25,26].
  - WES has provided additional confidence in the successful elimination of the virus in previously endemic countries (including in Nigeria, Egypt, India and others).

- **Settings at heightened risk of WPV1 reintroduction:**
  - Iran is at high risk of reintroduction of WPV as it is adjacent to endemic countries. WES has provided detection of WPV1 (in the absence of cases) with genotypic links to Pakistan as well as VDPV and provided reassurance of outbreak control[27].
  - Conversely, the last known WPV circulation outside Pakistan and Afghanistan was found through AFP surveillance in Malawi and Mozambique in 2021 and 2022. Neither had functioning WES programs at the time, but programs have since been established to enhance surveillance and exclude ongoing poliovirus circulation[28].

- **Following breach of containment of WPV:**
  - WES has been implemented following a suspected breach and provided additional evidence of successful containment in varied settings including the Netherlands[4].

- **Settings at heightened risk of cVDPV outbreaks:**
  - In Egypt in 2021, WES showed sensitivity to detect multiple separate incursions of VDPV with genotypic links to Sudan, Chad and Yemen, as well as a locally-emerged cVDPV outbreak following supplementary OPV campaigns[29].
  - In Madagascar, where there have been recurrent cVDPV1 outbreaks, sequencing of WES and clinical cases and contacts and phylogenetic analysis have shown the local emergence and spread of four distinct emergence groups characterized as recombinant between PV type 1 and other type B and C enteroviruses[30].

- **Settings at lower risk of poliovirus reintroduction (with routine use of IPV):**
  - Many polio-free countries that have adopted IPV use WES to monitor for the reintroduction of poliovirus and provide reassurance that it is not circulating silently[31–34, 37] .
  - In Europe in 2024, three countries (Germany, Poland and Spain) have had repeated wastewater detections of cVDPV genetically linked to a strain emerging in Nigeria, while no cases were detected[21].

- **Settings with outbreaks:**
  - Following initial detection, enhanced surveillance with additional sites, frequency of testing and rapid results has been shown to assist in characterizing and containing outbreaks[36–38].

- WES has been used to detect, characterize and genetically link a multi-country outbreak of cVDPV2 involving silent circulation in the UK (London and Northern Ireland), Canada and Israel and a single paralytic case in New York State[31,39–42].

- **Settings at risk due to conflict:**
  - Conflict and other complex disasters create heightened risk for poliovirus re-emergence, due to disruption of routine vaccinations, sanitation systems, as well as other factors. In Gaza, WES was able to continue during the 2023 conflict and provided early warning of cVDPV2 circulation [43].

There is an ongoing GPEI focus on expanding WES sites in priority countries and enhancing performance through a standardized monitoring framework, tools and support[16,44]. These tools address key process aspects such as completeness, quality and timeliness of sample collection, transport, testing and reporting culture and sequencing results as well as the sensitivity of enterovirus detection. By the end of 2023, 900 sites were reporting WES results from 86 countries into polio surveillance including 378 sites from 27 of the 28 GPEI 2023 priority countries. The priority country WES results have shown some improvement in site performance quality metrics; the enterovirus sensitivity target has been achieved by 58.8% of sites and 19 countries have > 80% of sites meeting the >50% enterovirus isolation rate[24].

4. WES objectives and related public health actions

The full integration of WES and clinical surveillance as part of the poliovirus eradication program, including contextual use of routine and agile WES, provides a model for integrated multimodal surveillance for other disease programs.

Use of WES enhances the timeliness and sensitivity of poliovirus detections and is relevant to countries which are poliovirus-free, currently WPV1 endemic, or which have experienced poliovirus incursion or outbreaks. In areas considered 'polio-free', a single instance of WPV or VDPV detection from WES triggers further investigation. Responses to WES poliovirus detection include further investigation in the catchment area of the WES site and repeated detections may trigger enhanced AFP surveillance and additional WES and supplementary vaccination campaigns.

4.1. Routine WES for poliovirus

WES supplements AFP surveillance in the following ways:

- Through the timely detection of WPV1 or VDPV importations and the emergence of circulating vaccine-derived polioviruses (cVDPVs).
- Through tracking ongoing transmission of polioviruses to guide vaccination strategies and provide evidence for the certification of disappearance of polioviruses from the environment.

Routine WES involves consistent sampling at the same sites using consistent methods

4.2. Agile WES for poliovirus

Agile WES with expanded and/or more frequent sampling is triggered by a clinical or environmental detection of WPV or VDPV during routine surveillance.

The response to a detection from a sewage sample (or an AFP case) is determined by the epidemiologic context, whether unexpected in a poliovirus-free setting or expected in an endemic or known outbreak. Agile WES is used to help characterize the extent or lack of community circulation, identify genetic and geospatial epidemiologic linkages as well as to inform and target the response and assess its effectiveness. Rapid implementation of an agile WES response is only feasible when there is an existing routine program.

Agile WES means that it is time-limited surveillance with a specific trigger to initiate. Agile WES involves establishing new time-limited activities or purposive changes in the existing WES program, e.g. sampling more frequently or in different locations, reducing the turn-around time to results, and/or performing new of different analyses.

4.3. Public health actions arising from WES for poliovirus

Public health actions arising from poliovirus WES depend on the context and are described in the GPEI eradication strategy 2022 – 2026[12] and the current Global Polio Surveillance Action Plan.

5. WES additional methodological considerations for poliovirus

This section should be read in conjunction with general methodological consideration in Section 5 of *Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration* (available here).

It has been over 20 years since the production of the first comprehensive guidelines for WES of poliovirus [14]. The guidance includes advice on sampling, analysis, and reporting. The information is reported via WHO and the GPEI. Examples of published guidance and seminal publications are given in the reference list[12,14–16,19,45].

There are five primary active areas of work in a poliovirus WES system:

- Drafting and validation of a national plan.
- Site selection and management.
- Sample collection and transport to the laboratory.
- Laboratory analysis (viral isolation, molecular methods and sequencing).
- Use of information for action.

5.1. Sampling methods

The strategy and criteria for selecting and monitoring sample collection sites for a poliovirus WES program, relevant to the poliovirus risk, population of interest and socioeconomic and political context, are described in detail in the 2023 field guidance[46]. These include considerations for site selection in settings with and without convergent sewered networks as well as performance monitoring to assess quality and discontinue non-performing sites. The guide also describes in detail the requirements for safe and high-quality sample collection, documentation, transport, and storage.

There are no special considerations for collecting environmental samples for poliovirus WES, beyond those used for conventional microbiological sampling for environmental monitoring and WES. Conventional grab samples, composite samples (time or location), and passive/trap samples, have all been successfully utilized. No method is perfect and all have pros and cons. Selection may be based on the specific context and use case. Periodic grab sampling is the method most widely used as a pragmatic feasible method. However, it has limitations in terms of cost, sensitivity and timeliness; it requires collection during a narrow window of time, has low temporal coverage and also requires costly transport of bulky water samples. Alternative improved sampling and concentration methods that address some of these limitations are available, such as filtration methods[47–50] but do themselves have other limitations. Other sampling innovations combined with near point-of-care testing are under evaluation.

5.2. Laboratory methods and interpretation

The most common, long-established WES tests for poliovirus include virus isolation using cell culture[12,14–16,19]. This detects all poliovirus types which are then identified as vaccine-like or wild type using an intratypic differentiation test (ITD) with a set of six rRT-PCR assays. Specifically, the most recent WHO guidance [19] and working draft [15] protocols use real-time RT-PCR to follow up culture-based testing. Selected specimens are then transported to GPLN-accredited specialized laboratories for sequencing to

further identify vaccine-derived polioviruses and confirm the presence of programmatically important polioviruses. However, these methods are not rapid; GPEI GPLN performance requirements are for end-to-end processes to be completed within 35 days. In all cases, laboratories analyzing concentrated wastewater in which poliovirus is detected (except Sabin-1 and -3) need to adhere to GAPIII protocols and transition to GAP IV protocols[51,52].

There is a strong desire to use direct rRT-PCR testing of AFP cases, noting the benefit of more timely and sensitive detection as well as consideration for use for wastewater or environmental samples. Although this is not yet recommended, if they are used on WES samples, WHO does provide guidance on how to report any positive results and provide samples to accredited laboratories [53]. Direct molecular methods for WES samples have been used in a variety of contexts to identify both wild type and vaccine-derived polioviruses, even though the negative predictive value is still suboptimal. Examples include successful use in the characterization and timely response and control of the multi-country cVDPV outbreak in USA (New York State), UK (London) and Israel, where both RT-PCR and sequencing were used[40–42,54]. Global specialized laboratories in the GPLN (WHO) are in the process of evaluating use of molecular methods and will include any changes in their periodic updated guidance.

5.3. Reporting and communication

The GPEI provides an interactive dashboard, integrating clinical case-based surveillance and WES results, with views tailored to the end user and geospatial and tabular displays[17]. These include AFP and WES poliovirus detections, molecular epidemiology and quality assurance metrics.

5.4. Acceptability of WES for poliovirus

Population-level WES is widely used globally as part of the poliovirus surveillance program. WES for poliovirus is aligned with the WHO ethical principles of public health surveillance, and WES is widely accepted and utilized globally for this purpose. The polio national action plan is the result of an inclusive process with the engagement of community, environmental and sanitation authorities and other stakeholders, so relevant acceptability and ethical considerations should be discussed and addressed in the tailored country action plan and during monitoring and evaluation. In WPV1-endemic countries where there are well documented acceptability issues in relation to poliovirus vaccination, the surveillance program is widely implemented and has had no such issues.

Cross-cutting acceptability, ethical, legal and related issues relevant to all WES activities are discussed in the WES overview document.

6. Integrated surveillance and multitarget WES considerations

6.1. Integration of poliovirus WES into existing surveillance and response

- WES is fully integrated together with clinical and other surveillance as part of the polio program, including contextual use of routine and agile WES. This provides a best practice model for integrated multimodal surveillance including WES for other disease programs.
- WES integration includes as part of planning for best fit combined surveillance as well as centralized support from the GPLN and data and information services for both clinical and WES activities. Visualization of both WES and clinical surveillance results together supports timely decision making.
- However as the GPEI goal is eradication it has remained to date largely a vertical program, In its current end stage strategy, the GPEI is seeking to link and strengthen the overall disease surveillance and response systems in target countries[55].

6.2. Integration of multi-target WES surveillance together with poliovirus

- At the end of 2023, the polio program had 900 sites across 86 countries concentrated in low- and middle-income countries, a projected continuation of WES for 10 years after eradication is achieved, well established WES procedures, infrastructure and partners and a commitment to integrate priority WES activities (e.g. SARS-CoV-2). It also has a commitment to innovate and strengthen sensitive and timely sampling and analysis methods.
- Well-established poliovirus WES programs can provide local capacity and capability from which to expand to other pathogens at low marginal costs for routine and/or agile WES responses. This is dependent on continued financial and logistical support.
- Multi-target surveillance leveraging polio programs has potential to enhance equitable surveillance with epidemic/pandemic preparedness and response capability, given the distribution of polio sites in lower-income countries.
- Distribution of polio sites in Africa, Asia and tropical and semitropical zones may overlap with those of other pathogens of interest, such as fecal-oral pathogens S. Typhi, S. Paratyphi A and B, and Vibrio cholerae as well as monkeypox virus and various mosquito borne arboviruses among others.
- Beyond this, in principle, any pathogen or its genetic material shed via pathways that enter sewage and human-influenced environmental water (e.g. via stools, urine, secreta, skin, or blood), can potentially be detected in samples collected for the poliovirus WES program.
- Therefore, opportunities to leverage and integrate other pathogens with the existing or slightly modified poliovirus WES programs, while strengthening or not undermining polio WES are likely to be substantial. However, there are known limitations and challenges. The poliovirus WES program has standardized methods - covering sample program design, sample site selection and validation, sample collection and transport, analysis, and reporting -which may not be optimal for other potential targets. This may limit the flexibility to adapt the poliovirus WES program in ways that support multiple pathogen WES.
- Conversely, existing WES activities for other pathogens may be leveraged in part to integrate poliovirus – for example for more cost-efficient sampling and transport.

7. Key knowledge gaps and applied research priorities

The polio effort is at a critical juncture, with the scale and speed of disease spread since the COVID-19 pandemic threatening to jeopardize the end-stage phase of eradication. There are several applied research priorities to optimize application of WES for polio and strengthen polio surveillance. Key knowledge gaps and recommended areas of applied research include:

- The extended GPEI 2022 – 2029 Strategic Plan identifies the need for new tools, approaches and partners to optimize the effectiveness of polio WES surveillance methods. This includes system attributes of sensitivity and timeliness which are feasible to implement at scale in relevant and varied endemic and high-risk global settings (including key geographic regions prone to cVDPV outbreaks, a high proportion of which are low-resource settings with low coverage of improved sanitation systems in hot tropical climates).

- Applied research is underway including in areas covering:
  - Innovation, validation and standardization of sampling methods. This includes improved sensitivity and temporal coverage of periodic grab sampling[47–49].
  - Laboratory methods, including those with direct molecular detection and sequencing. This includes improved sensitivity and timeliness of culture, with lower biosafety requirements (i.e. direct molecular methods don't involve cultivation of live viruses)[40–42,54,56–58].
  - Other capacity building and system program quality improvements to shorten time to result and time to effective response in support of the eradication end game.

- Research to understand the trade-offs that are necessary when developing methods and optimize the contextualized decisions. The perfect method does not exist, and purposive contextual trade-offs are necessary to achieve surveillance objectives.

- Research to explore the optimization of cost-effective, sustainable polio WES that continues beyond eradication for continued monitoring. This is likely to include identification of other targets for multitarget WES, while ensuring that core poliovirus WES effectiveness is maintained or enhanced.

References

The general information on poliovirus was drawn from the publicly available GPEI, US CDC and WHO open-source guidance, which should be consulted for the most current information[17,59,60].

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