POLIO GLOBAL ERADICATION INITIATIVE

Field guidance

for the implementation of environmental surveillance for poliovirus

EVERY LAST CHILD

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ISBN 978-92-4-005754-8 (electronic version)
ISBN 978-92-4-005755-5 (print version)

Published by the World Health Organization (WHO) on behalf of the Global Polio Eradication Initiative

Suggested citation. Field guidance for the implementation of environmental surveillance for poliovirus. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at http://apps.who.int/iris.

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Acknowledgements

This document reflects contributions from epidemiologists, laboratorians, information system specialists, public health and gender experts, from field, regional and global levels, in a process led by agency partners of the Global Polio Eradication Initiative (GPEI): Rotary International, the World Health Organization (WHO), the US Centers for Disease Control and Prevention (CDC), the United Nations Children's Fund (UNICEF), the Bill & Melinda Gates Foundation, and Gavi, the Vaccine Alliance.

Acronyms and abbreviations

AFP	Acute flaccid paralysis
BMFS	Bag-mediated filtration system
CO	WHO country office
cVDPV	Circulating vaccine-derived poliovirus
ES	Environmental surveillance
ESIWG	Environmental Surveillance Implementation Working Group
EV	Enterovirus
GIS	Geographic information system
GPEI	Global Polio Eradication Initiative
GPLN	Global Polio Laboratory Network
GPS	Geographic positioning system
IPV	Inactivated polio vaccine
ITD	Intratypic differentiation
MOH	Ministry of Health
NPAFP	Non-polio acute flaccid paralysis
NPEV	Non-polio enterovirus
ODK	Open data kit
OPV	Oral polio vaccine
PEF	Polio-essential facility
PPE	Personal protective equipment
PV	Poliovirus
RO	WHO regional office
SIA	Supplementary immunization activity
SL	Sabin-like
SOPs	Standard operating procedures
VDPV	Vaccine-derived poliovirus
VDPV2	Vaccine-derived poliovirus type 2
WHO	World Health Organization
WPV	Wild poliovirus
WPV1	Wild poliovirus type 1

About this guidance

Environmental surveillance for poliovirus plays an important role in the efforts to achieve and maintain a polio-free world. Through the examination of human faecal samples from untreated wastewater collection systems, ES provides valuable information on the presence or absence of poliovirus circulation in defined geographical areas. The following field guidance was developed by the Global Polio Eradication Initiative (GPEI) to support national polio eradication programmes in initiating and implementing high-quality and highly sensitive environmental surveillance (ES) systems.

The primary purpose of this document is to expand on prior guidance on the programmatic and operational aspects of environmental surveillance for polioviruses, focusing on site selection, sample collection and transport, and the use of data for action. In so doing, this document will provide country programmes supported by the GPEI with detailed guidance on the preparation and implementation of polio environmental surveillance. Other documents, such as the 2015 *Guidelines on environmental surveillance for detection of poliovirus*, contain information on laboratory procedures for testing environmental samples for detection of poliovirus.

This guidance is intended for use by individuals and organizations involved in polio eradication efforts that include: polio surveillance officers, managers, laboratorians, immunization programme managers and officials, and technical staff from GPEI partner agencies involved in supporting and monitoring poliovirus surveillance activities.

Introduction

Environmental surveillance (ES) plays an increasingly important role for the Global Polio Eradication Initiative (GPEI) in its efforts to achieve and maintain a polio-free world. Through the examination of composite human faecal samples from untreated wastewater collection systems typically located downstream from high-risk populations, ES provides valuable information on the presence or absence of poliovirus circulation in defined geographical areas. Though it cannot link poliovirus directly with infected individuals, it enhances the sensitivity of surveillance for acute flaccid paralysis (AFP) and can provide an early warning indicator on potentially hundreds or thousands of silent polio infections during an outbreak or in an endemic area.[1]

Field guidance for the implementation of environmental surveillance for poliovirus complements and updates previous ES guidance.[1,2,3,4] While other documents focus on updating laboratory procedures for testing environmental samples for detection of poliovirus, the primary purpose of this document is to expand on prior guidance on the programmatic and operational aspects of ES, focusing on site selection, sample collection and transport, and the use of data for action. In so doing, this document will provide country programmes supported by the GPEI with detailed guidance on the preparation and implementation of polio environmental surveillance.

This guidance is offered in support of two overarching goals:

(1) to provide operational and programmatic guidance for field implementation of ES; and

(2) to provide tools and resources to facilitate high-quality data collection, analysis and monitoring.

Well-implemented ES can significantly increase the sensitivity of surveillance for poliovirus in certain areas. ES has been used for many years to detect and monitor the reintroduction of wild poliovirus (WPV) into polio-free countries (such as Finland, Netherlands (Kindgom of the) and Israel), [5] as well as to provide confidence in the successful elimination of the virus in previously endemic countries (including Egypt and India). [6,7,8] Repeatedly, ES has detected polio transmission in areas where no virus-positive AFP cases were found, [9] a feat which highlights ES as a powerful complementary, sentinel-based disease detection system.

The biological basis of environmental surveillance

As a surveillance method, ES follows the basic pathogenesis of poliovirus. Humans are the only reservoir for poliovirus, the fundamental characteristic which qualifies polio for eradication. Transmission of poliovirus occurs from person-to-person via faecal-oral or oral-oral routes. An infected individual, irrespective of the presence of symptoms, will experience poliovirus replication in the nasopharynx for a few days and in the intestine for several weeks,[10] which causes viral shedding into the environment

that can then be detected through sewage and wastewater. Shedding can be intermittent, and the amount of virus excreted into the stool will vary depending on the individual and time of infection; between zero and 10e8 polioviruses per gram stool [11] with an average peak level shedding of 10e5 per gram stool.[12,13]

Factors that affect transmission of the virus include the extent of crowding, hygiene levels, water quality and sewage handling. In endemic countries and areas in general, WPVs have a distinct seasonal

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The case-to-infection ratio of polio drives the need for highly sensitive surveillance systems. Wild poliovirus type 1 (WPV1) has a paralytic rate of one case for every 200 infections (1:200), whereas vaccine-derived poliovirus type 2 (VDPV2) has an infection rate of one case for 2,000 infections (1:2000). Particularly as country programmes target VDPV2s that have a 90% lower infection rate, ES can support early detection and enable rapid response to interrupt a potential outbreak.[1]

pattern of circulation (in contrast to the Sabin-like [SL] vaccine viruses from the oral polio vaccine [OPV]) that varies by geographic area. In tropical and semitropical areas, circulation tends to be year-round or often associated with the rainy season. In temperate areas prior to poliovirus immunizations, polioviruses were mostly prevalent in the summer and fall, although outbreaks could continue into the winter. Now in countries with routine use of OPV, SL virus should be detectable at all times, or detections may cluster around the time of national or subnational supplementary immunization activities (SIAs).

The Global Polio Laboratory Network (GPLN) has developed and standardized sensitive methods to concentrate sewage water samples and test them for the presence of poliovirus and then further differentiate wild polio from vaccine-derived poliovirus (VDPV) or SL virus particles. Molecular genetic sequencing can then link polioviruses isolated in other environmental samples or in stool samples from AFP paralytic cases to confirm poliovirus circulation and track routes of transmission. *(1,3,14)*

The probability of detecting poliovirus in wastewater samples will depend on a number of variables, such as the duration and amount of poliovirus shed by one or more infected individuals in the catchment area,*(7,15)* the effect of physical and mechanical factors on the dilution and survival of poliovirus in the sewage system, *(9)* the location of the excreter relative to the sample site,*(3)* the frequency of collection and the laboratory's ability to detect existent poliovirus, *(1,9)* and seasonal variation in enterovirus isolation. As such, environmental surveillance might not be feasible for implementation in all desired locations.

The role of environmental surveillance to polio eradication

To monitor progress towards wild poliovirus type 1 (WPV1) eradication in the last remaining regions with community transmission and to detect VDPVs wherever these may emerge, highly sensitive surveillance is essential. Poliovirus surveillance continues to rely largely upon 'gold standard' surveillance for cases of AFP, but ES plays a highly critical role. On the one hand, ES can be more effective at detecting poliovirus circulation than AFP surveillance in some settings, such as in areas with suboptimal AFP surveillance or in populations with high vaccination coverage or with inactivated polio vaccine (IPV) in essential immunization schedules, as the paralysis-to-infection ratio will be very small in these contexts. In such settings ES is likely to reduce the time to detection of circulation by several months *(16)*. On the other hand, the lack of convergent sewer networks in rural areas and some urban settings in developing countries reduces the feasibility (and/or cost effectiveness) of ES, thus reducing its advantage over AFP surveillance in some areas at high risk for poliovirus circulation.

Therefore, to maintain poliovirus surveillance at the high sensitivity and specificity levels required to achieve and certify eradication, countries may rely on a combination of environmental and AFP surveillance, implementing best practices that optimize their effectiveness in the field.*(17)*

More specifically, ES can supplement AFP surveillance in the following ways:

a) through the timely detection of WPV1 or VDPV importations and the emergence of circulating vaccine-derived polioviruses (cVDPVs); and

b) through tracking ongoing transmission of WPV1 and cVDPVs to guide vaccination strategies and provide evidence for the certification of disappearance of poliovirus (Sabin, WPV and VDPV) from the environment.

Since 2013, the GPEI supported ES in five countries (Afghanistan, Angola, Kenya, Nigeria and Pakistan). Under the *Polio Environmental Surveillance Expansion Plan* (PESEP),*(18)* starting in 2016, the GPEI began increasing the number of sampling sites in countries that were then endemic (Afghanistan, Nigeria and

Pakistan) and initiating ES implementation in six new countries (Burkina Faso, Cameroon, Chad, Guinea, Madagascar and Niger). By the end of 2019, 26 additional countries had initiated ES under the PESEP. Currently, the programme supports approximately 550 ES sites for poliovirus in 45 countries; with over 12,000 ES specimens processed annually by the GPLN. As the global epidemiologic situation evolves, rationale expansion and optimization of existing ES networks to ensure sensitivity of the system, will be a priority of the programme.*(19)*

Three principles for environmental surveillance implementation

Each country looking to initiate a new ES system or enhance an existing system should prioritize three fundamental principles that have helped the polio eradication programme achieve notable successes in the rapid detection of polioviruses in the environment. These principles should guide programme efforts when a country starts to locate and validate new sampling sites, collect and transport samples, or report on and evaluate site performance, as they help with achieving and maintaining high-quality surveillance.

The three principles for successful implementation are: (1) understanding key areas of risk or vulnerabilities that can make a population susceptible to WPV1 or VDPVs; (2) balancing the drive to increase a surveillance system's sensitivity while ensuring its feasibility with respect to funding, resources and staff; and (3) ensuring a sustained collaborative effort from frontline field sample collectors and programme focal points to laboratory personnel.

1. Understanding risk prioritization

The function ES fulfills in a country will vary depending on the epidemiologic situation of polio eradication and activities of the polio immunization programme, such as the exclusive use of IPV or schedules that include IPV with rounds of OPV. See **Table 1**.

Table 1. Role of environmental surveillance based on epidemiological situation

Epidemiology	Role of environmental surveillance
Endemic countries	• Monitor genetic diversity of poliovirus and differentiate circulation associated with indigenous strains versus re-introduction from other areas. • Supplement AFP surveillance and identify epidemiological links between separate geographical areas, potential virus reservoirs and transmission routes. • Document the elimination of indigenous WPV.
Areas with recent poliovirus circulation	• Document the scope of virus transmission to guide immunization activities. • Document effectiveness and impact of vaccination campaigns in outbreak response. • Confirm the end of an outbreak through the disappearance of VDPV or WPV from the environment in conjunction with negative isolation of poliovirus in stools from AFP cases.
Polio-free countries	• Detect the re-introduction of WPV or an emergence of cVDPV, especially in settings with non-existent or suboptimal AFP surveillance and/or use of IPV. • Document poliovirus released from manufacturing facilities or laboratories. • Monitor the disappearance of Sabin strains following OPV withdrawal.

AFP= acute flaccid paralysis; cVDPV= circulating vaccine-derived poliovirus; IPV= inactivated polio vaccine; OPV= oral polio vaccine; VDPV = vaccine-derived poliovirus; WPV= wild poliovirus

For countries supported by GPEI, the allocation of resources to for ES implementation uses a prioritization scheme with distinct parameters for assessing the risk for poliovirus circulation at the national and subnational level. See **Table 2**.

Table 2. Risk categories that inform GPEI country prioritization

Risk of WPV1 transmission	Countries are classified into risk categories based upon the existence of endemic circulation or their proximity to a country with circulation: high, medium and low.
Type 2 cVDPV emergence and transmission risk	Countries are ranked based upon the presence of a current outbreak and/or risk of importation from a neighbouring country with an outbreak or the risk of VDPV emergence within the country.
Population immunity risk	National coverage with three doses of diphtheria, tetanus toxoids and pertussis (DTP) vaccine is used to estimate a risk of emergence or transmission of poliovirus due to population immunity.
Surveillance risk	The quality of a country's AFP surveillance system is assessed and ranked by its achievement of non-polio AFP (NPAFP) rates.
Containment	The presence (or absence) of a polio-essential facility (PEF) where type 2 (WPV2/VDPV2 and OPV2) and type 3 (WPV3/VDPV3) poliovirus manipulation will occur provides determination of the risk of a containment breach.

AFP= acute flaccid paralysis; cVDPV= circulating vaccine-derived poliovirus; VDPV= vaccine-derived poliovirus; WPV1= wild poliovirus type 1

Within each country, environmental sites should be located in areas with populations at high risk for poliovirus circulation, which will vary by country (see **Site management** below).

Countries not receiving funds directly from the GPEI for poliovirus surveillance may have different criteria for the introduction of poliovirus ES, especially if it is integrated with surveillance for other pathogens, such as enterovirus or cholera. Feasibility of implementing an effective ES system relies on availability of good sampling sites, trained personnel and funding. All countries need to balance the effort and resources required by ES for poliovirus against their own risk of poliovirus circulation with the extra detection sensitivity that ES may or may not add to their current poliovirus surveillance systems, as explained below.

2. Balancing sensitivity and feasibility

An objective assessment of an environmental site's sensitivity is based on the isolation of enterovirus from collected samples, which generally determines that the site is functional for the detection of poliovirus. While there are many ways to increase the sensitivity of a surveillance system, not all may be feasible because of limited time, resources and staff. The best approach to achieving high-quality surveillance is to balance sensitivity with feasibility through a targeted approach. While AFP surveillance targets the entire country population, ES will usually be a sentinel surveillance. The potential yield of sampling sites and their epidemiologic significance will vary significantly from country to country – and even within the same country.

It is important to address the following considerations when selecting new ES sites:

(1) target the appropriate population (i.e., populations at risk of re-introduction, emergence or transmission);

(2) select a collection point where with converging sewage from a population large enough to have several individuals shedding poliovirus for several weeks;

(3) minimize or eliminate factors that may interfere with poliovirus detection during sample collection, transport and testing; and

(4) carefully assess laboratory capacity and resources when identifying the testing laboratory, site location, sampling schedule and overall number of sampling sites.

These considerations will be detailed in steps outlined below (under **Environmental surveillance field activities**) to help country's plan and operationalize an ES system for poliovirus.

3. Ensuring a sustained collaborative effort

Environmental surveillance brings together both field and laboratory staff and incorporates collection of sewage instead of clinical specimens from patients, therefore a collaborative effort across the country, regional and global levels is required, to be successful.

Among the expertise needed to plan and maintain robust ES systems are:

- epidemiologists, local public health officials in both polio surveillance and immunization programmes;
- WHO staff at the country and regional level involved in polio surveillance and immunization;
- local sanitary engineering authorities and technical staff involved in maintenance of wastewater and sewage infrastructure at the national and municipal or district level; and
- staff from the GPLN polio laboratory who will be involved in processing samples for polio surveillance.

Environmental surveillance field activities

A national environment surveillance plan should be developed and integrated into the overall national plan for poliovirus surveillance for all countries implemented ES. Key elements for the inclusion of ES field activities into the national surveillance plan and/or the development of a national ES plan are outlined in **Annex 1.**

ES planning should start through an in-country initiation mission that draws upon expertise at the national and subnational levels, including the Expanded Programme on Immunization (EPI), national polio laboratory (NPL), local sanitation engineering authorities and other relevant provincial and local authorities. The WHO regional office should also be consulted early in the planning process. With support from this broad range of stakeholders, the country programme will identify at-risk populations and areas, locate environmental sites, define existing resources and future needs for sample collection and transport, create an implementation timeline and sampling schedule, and provide a breakdown on operational costs for each selected site. A sample agenda for the ES initiation mission is provided in **Annex 2**.

Steps toward ES field implementation

1. **Develop or update** the National Environmental Surveillance Plan (see Annex 1).
2. **Select areas** with populations at highest risk for importation or enabling transmission following importations of WPV1/VDPV, or for emergence of cVDPVs.
3. **Select sampling sites** based on:
    a. convergent wastewater network for a sample large enough of at-risk populations;
    b. absence of toxic pollutants; and
    c. accessible for collection and transportation.
4. **Assess laboratory capacity** and the logistics and resources needed for the timely transport of samples to the lab.
5. **Develop a plan** for regular interpretation and reporting of laboratory results.
6. **Coordinate** ES with other surveillance systems for poliovirus.

There are three primary areas of work in an ES system: (1) site management; (2) sample collection, transport to the laboratory, and analysis; and (3) the use of information for action. Each are elaborated below.

1. Site management

Site management entails the full life cycle of sampling sites: from selecting and opening, to operating and monitoring, and to closing when deemed necessary. The number of environmental sites in-country may vary from a handful of sentinel sites in large cities that are “population hubs”, to 50 or more sites in countries with well-developed ES networks (e.g., Pakistan, Nigeria). In addition to ensuring documentation of the location and characteristics of each ES site with the country and global polio network, polio eradication programmes should supervise sample collection, transportation operations and data provided by all sites to monitor their effectiveness and programmatic relevance.

1.1 – Opening an environmental site

Optimal areas for environmental surveillance

Environmental surveillance should be conducted in areas that will support and strengthen country polio surveillance efforts. Optimal locations in-country can be identified by mapping vulnerable populations and geographic areas that either pose a risk for poliovirus circulation or present an opportunity for gaining access to previously inaccessible and highly mobile communities. The appropriate location of ES sites plays a major role in its reliability to detect the absence or presence of poliovirus circulation.

The following criteria will facilitate locating new ES sites for optimal performance:

- Areas with populations at epidemiologic risk for poliovirus circulation based on:
    - a history of WPV or VDPV transmission; and
    - a shared border with areas or countries with recent endemic or outbreak transmission.
- Areas with suspected immunity gaps due to inadequate access to vaccination (i.e., minorities, temporary workers, undocumented migrants) or high numbers of vaccination refusals.
- Communities with suboptimal access to sanitation and health care, such as slums, illegal urban or peri-urban developments, and areas with a high proportion of minoritized groups.
- Areas with suboptimal AFP surveillance indicators and areas with orphan viruses.
- Camps and host communities for refugees or internally displaced populations, especially if they are fleeing from areas with current or recent history of poliovirus circulation.
- Hubs for transportation, commerce or large gatherings (i.e., festivals, markets and pilgrimage sites) with presence of women and infants.

Selection of specific areas for location of environmental sites should be based on the country's polio risk assessment, epidemiological situation and the expected role of ES in the broader context of national poliovirus surveillance.

Types of environmental surveillance sites

There are two types of surveillance sites: permanent or routine sites and temporary or ad hoc sites (see **Table 3**). Changes in the number of sites and their locations are expected and accounted for through multi-year planning produced in close engagement with public health officials from agencies within the GPEI and within the country.

Table 3. Types of environmental sites

Site type	Role within the national plan
Permanent or routine sites	Samples from permanent sites are expected to be routinely collected, and at minimum, on a monthly basis (i.e., 12 sample-months per year). Following a six-month period (approximate), new sites may be considered for continuation as a permanent site.
Temporary or ad hoc sites	Sites selected to enhance surveillance in areas considered temporarily at high risk of poliovirus circulation because of an outbreak or sudden influx of an at-risk population. The programme should expect to stop collection of samples from these sites within a few months or years, once the outbreak has been controlled. Some sites may be open only during select times of year ("**seasonal**") in which case the expected sample-months per year are site specific.

1.2 – Selecting sampling sites

Once areas of epidemiological interest have been selected within the country, workshops and field visits will be necessary to identify sampling points where sewage collection will be both feasible and cost-effective.

A feasibility assessment should consider the factors elaborated below.

Catchment population

The number of people living in the catchment area of an ES site (i.e., those connected to a converging sewer network) affects the sensitivity of poliovirus detection in a population. In general, a catchment population of ~100 000 to 300 000 individuals for a sampling site is recommended as the optimal size to allow isolation of poliovirus if it is circulating in the population. As not all individual households may contribute faecal material to draining wastewater flow or systems, it may be necessary to identify areas with larger overall populations.

A sampling site is considered **sensitive** when enteroviruses (polio or non-polio viruses) are detected in at least 50% of samples.

- While a **large catchment population** may allow monitoring of more people with fewer samples, it also decreases the capacity to detect small numbers of poliovirus excreters, as the increasing number of non-excreters may dilute the virus to below the limits of detection (1 $CCID_{50}$ per 1.5 ml of waste water is the minimum requirement for cell culture).

- A **small catchment population** might miss or delay detection of circulation in the area since (1) the number of infected individuals shedding poliovirus is often too small (or absent) to be detected at the time of sampling; and/or (2) the number of susceptible individuals is too small to sustain circulation long enough to be detected in samples that, for a cost-effective surveillance, should occur once or twice a month [2]. Sampling small catchment populations would therefore require many sites to be sampled to cover a significant proportion of a large population. With small catchment populations, the programme may opt instead for '**composite samples**' whereby similar sized samples are collected from several sites and the laboratory may test them together as a composite sample.

Cities and other urban-like settings such as transit hubs should receive focus to determine feasibility for ES sampling. A rapid assessment can determine whether ES might be suitable for a particular city or urban area and may be used as an early step in the overall implementation process (see **Annex 3**).

Type of sewer systems

When identifying a sampling site, the national programme should consult with both local sanitary engineers and epidemiological experts who can assist in evaluating sewer and wastewater systems in the area and provide information of the catchment populations. Below are types of sewer systems. Further examples of wastewater treatment facilities and poor or sub-optimal sites are provided in **Annex 8**.

- **Closed, converging sewer networks** that connect to household water closets are optimal for systematic ES. The best location for sampling sites is the inlet closest to the entry into the treatment

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An average of one poliovirus excreter could be detectable in a sewer system covering up to 10,000–15,000 individuals, therefore small site ES may be relevant at *specific locations* such as camps or host communities for refugees or internally displaced populations where the suspicion of PV circulation is particularly high.2

plant, where the wastewater containing human faecal material from a larger population can be caught. However, for treatment facilities capturing large metropolitan areas, the catchment area may be segmented by moving the sampling point up-stream to inlets close to pumping stations or major collector pipes to ensure a target population of an acceptable size of ~100 000–300 000. A detailed epidemiological analysis of the population in each segment can guide the selection of few segments with representative groups at risk for poliovirus transmission.

Image 1a. Closed sewerage system.
Photos courtesy of H. Abdullahi, WHO

Image 1b. Closed sewerage system.

- **Open canals or water channels** may be the only choice available in developing countries. Before selecting the sampling point, it is important to conduct a thorough exploration and mapping of the wastewater drainage in collaboration with local sewer engineers.
    - Exploration is needed to detect blockages or obstruction of wastewater lines that may exclude segments of the catchment population, as well as to identify potential sources of toxic waste in the wastewater.
    - If comprehensive sewage network maps are not available, global positioning system (GPS) coordinates along the wastewater ways will allow the creation of “blue line maps” that may be modeled with specific computer software to estimate the catchment population for a specific sampling site. (See **Section 3, Use of information for action**).

Image 2a. Open drainage.
Photos courtesy of H. Abdullahi, WHO

Image 2b. Open canal.

In areas where human waste is disposed into **latrines, septic tanks or open fields** without a convergent system, environmental sampling is not recommended because the number of individuals disposing waste in a certain location is too small. Sampling in sewage treatment facilities where trucks dump raw sewage from septic pits and latrines is also not recommended as the catchment population is probably small and impossible to estimate, and access to the sewage facility usually depends on payment of disposal fees not on geographical location. Furthermore, sampling from sites that lack sufficient wastewater flow may result in lower surveillance sensitivity due to a variety of undefined or uncontrolled factors, such as environmental inactivation of viruses.

Toxic compounds

Wastewater channels with potential drainage of toxic compounds, should be avoided when selecting sampling sites as several kinds of biological and chemical compounds can reduce poliovirus survival.

- High temperatures, exposure to ultraviolet sun light, high ammonium concentrations, low pH and bacterial enzymes are major natural factors that inactivate poliovirus in wastewaters, whereas virus adsorption to sewage silts and solids have a protective effect.(7)
- Formaldehyde, bleach and other industrial waste may inactivate poliovirus and/or cause toxicity in culture cells.

Image 3. Environmental site with probable contamination from chemicals.
Photo courtesy of M. Bello, WHO

To determine if toxicity is present, observe the wastewater color and (lack of) odor at the sampling site, which may indicate the presence of toxic materials, such as:

- presence of a high amount of non-organic debris from daily life;
- presence of oil or high amount of foam on the water surface;
- presence of red, yellow or other colors not usually associated with presence of human waste; or
- chemical smell (e.g., chlorine, gasoline, motor oil) in areas of the wastewater.

As color and odor may be affected by other variables, in cases where wastewater is located near agricultural or industrial activities (such as dairy farms, factories, garages or cloth dying sites), the sample collection point should be moved up-stream, to avoid possible contamination by toxic waste .

Strategies for detecting probable contamination of a sampling site have limitations. The use of water color and smell to identify toxic compounds depends on the observational abilities and experience of the sample collector, supervisor or other professional. Additionally, the presence of toxic compounds may be intermittent or absent during the site field assessment. Field officers must therefore be vigilant in regularly monitoring for potential toxicity.

At the time of writing this guidance, the use of water quality probes to support selection and monitoring of sites for poliovirus ES is under evaluation. Most of the available electronic probes and field water tests are not applicable to detect the presence of substances that may interfere with poliovirus survival in wastewater or interfere with laboratory testing protocols; rather, they detect variables or compounds associated with unsafe drinking water or recreational use. However, certain measurements, such as pH and total dissolved

solids (e.g., in Nigeria), have been associated with higher likelihood of enterovirus survival and should be further evaluated for field applicability.*(20)*

Accessibility

It is also important to assess transportation logistics and accessibility, when selecting environmental surveillance sites, as collectors will need to walk and stand in public areas for approximately 30 minutes or longer to complete procedures. Because most sampling is recommended early in the day to coincide with peak toilet use, access to the site should be confirmed during the early morning hours. Areas that are intermittently inaccessible because of flooding or other seasonal considerations should only be considered for ad hoc or temporary environmental sites and only in specific situations, such as an outbreak.

To support collector access, community leaders and authorities should be informed about the purpose of sample collection and the expected sampling times to avoid suspicion of potential illegal activities. Permission from the public or private authorities in charge will also need to be obtained in advance for sampling sites located in sewage treatment plants.

Under no circumstances should sampling sites be placed in areas with dangerous terrain, active conflict or where collectors could be exposed to life-threatening violence.

1.3 – Setting a sample collection schedule

The recommended collection time for sampling is always site-specific and should be determined as part of ES initiation and the site selection process. The collection time for each sampling site is decided after discussion with local sanitary engineers and on-site observation of the wastewater flow at the point of collection at different times of the day during the initial assessment. The sampling schedule must be discussed and agreed upon with the poliovirus laboratory.

- **Collection date:** Schedule collection dates to make the most efficient use of transportation and laboratory resources. For example, several sites may collect on the same day of the month or in consecutive days during the week so samples can be shipped in batches to minimize costs. Samples should arrive on a schedule that facilitates integration into the laboratory workflow. Advance notification and communication of potential changes in schedules are also important to avoid unnecessary delays in testing and reporting.
- **Optimal time of collection:** Generally, samples collected during the early morning hours of peak toilet usage (e.g., 06:30–08:30h) are more likely to detect poliovirus. The exact timing of the peak sewage flow through a sampling time will vary depending on the distance from the sampling point to the catchment population and the slope of the waterways (i.e., 30 minutes to several hours). Observation of the flow for several hours on more than one day will be useful to determine the optimal collection time for each collection point.
- **Sampling frequency:** The minimum sampling frequency is monthly for routine sites. The decision to increase sampling frequency (i.e., from once to twice monthly) needs to balance the potential enhancement in sensitivity or timeliness of detection with the increase in workload for the laboratory.

Sample schedule and time of collection

- The recommended collection time is site-specific.
- The recommended minimum frequency of sampling for routine or permanent sites is once per month.

Pooled or composite samples: Although 24-hour pooled or composite samples made from aliquots collected hourly would ideally be a more representative sample, it is expensive and may not be feasible for sampling points located in open sewage canals with open public access. If resources allow for composite sampling for a catchment area, collection times will need to be adjusted to account for travel time between sampling points. In the case of composite sampling inside a sewage treatment plant, agreement with staff in charge of the plant will be needed to confirm the best time to reach the peak flow at the inlet of the plant and entry access.

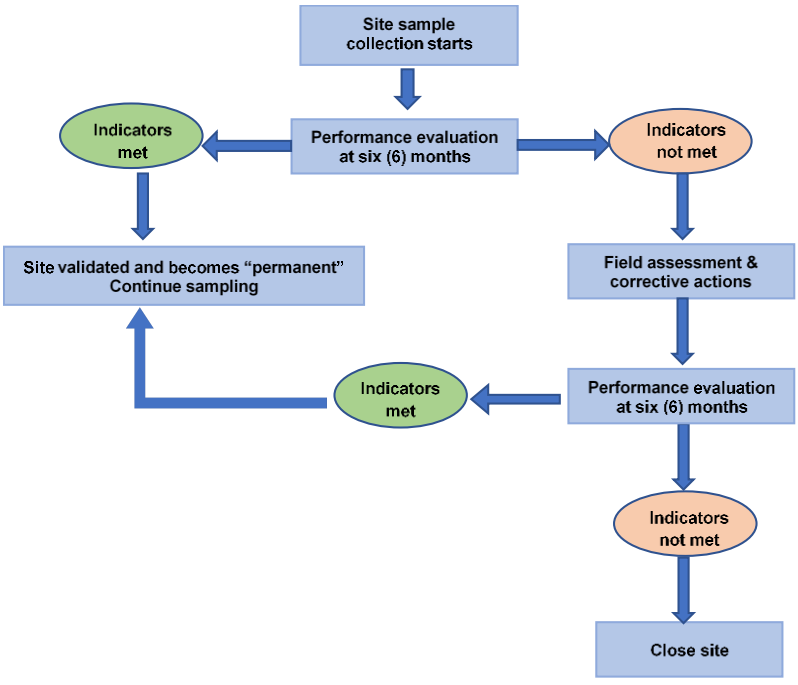
Ideally, selected sites should have the ability to support sewage collections year-round to account for the seasonal variation of enterovirus isolation in the environment; however, in some circumstances sites with limited duration of collection may be warranted (see **Table 3**, page 8).

1.4 – Validating an environmental site

The GPEI recommends that new environmental sites undergo a validation period of six to 12 months before formal inclusion in the network as a routine or permanent site. This period of validation ensures programme resources are aligned with sites that reach key performance indicators for sensitivity (see **Table 5** under "Monitoring and evaluating performance" in Section 3 below).

As shown in **Fig. 1**, validation involves a performance analysis during the initial six months after a site is opened. If the analysis suggests that the site is performing well, the site can be included in the national ES network as a permanent site. If performance indicators, especially enterovirus isolation rates, are below expectations, a field assessment and implementation of corrective actions will be conducted, followed by an additional six-month observation period if needed. If after additional observation, performance of a site does not improve, the site should be closed. A new site should be identified in the same catchment population if feasible and if the population is very relevant for the sensitivity of the surveillance network. If a new site in the same area is not feasible, an alternative site in other areas or populations can be considered.

Fig. 1. Algorithm for validation of a new environmental site



1.5 – Closing environmental sites

Whether linked to underperformance or optimization of the environmental surveillance network within a country, the decision to close a sampling site should be triggered after thorough investigations and discussions that involve the WHO regional office. Standard criteria and a decision-making process, as explained below, should be followed.

Criteria for closing a sampling site

Factors that affect the performance of a sampling site are multiple and often difficult to assess.*(1)*

- The site may no longer meet programme needs, or the initial reason for opening the site is no longer valid.
  - Country- or city-specific risk-assessment has strongly suggested that the risk profile has changed, and the ES site no longer represents a catchment population considered at-risk.
  - There is higher risk elsewhere in the country (prioritization).
- The sampling site shows poor performance for at least six consecutive months:
  - with no cause identified (e.g., seasonal variation) or suspected during on-site investigations or assessment; or
  - with no improvement in performance after corrective actions have been implemented (i.e., correct time of sampling, adequate reverse cold chain and arrival in the laboratory in good condition).
- Limitations in ES processing capacity may require rationalization of the ES network.

Operational framework and decision-making process for closing a site

Within each WHO region, countries should adhere to the following guidance for assessing underperforming sampling sites and proceeding to their closure. Assessment is under the responsibility of the national programme, which should discuss the proposed closure with the WHO country office (CO) and consult with the WHO regional office (RO) before taking the decision to discontinue sampling.

For any of the scenarios above, the following decision-making process must be systematically followed:

(1) The national programme documents the need to close one or several sampling sites that will be shared with the WHO RO and (as needed) WHO headquarters. The proposal (e.g., interim findings from an external monitoring visit) should be prepared in close collaboration with the WHO CO and include the rationale and timeline for the site(s) closure.

(2) GPEI advice may be requested on an ad hoc basis; recommendations will be sent back to the country within the week. A site opened in response to an outbreak should be closed in consultation with the lead of the outbreak response.

(3) The WHO CO and RO and headquarters may hold a final discussion and will inform all stakeholders about the decision through a short summary report.

(4) When closing a site, the RO should be informed, and the site data form (electronic, paper-based) updated to reflect the new status in the environmental site database.

2. Sample collection, packaging, and transportation to the laboratory

Grab sampling is the method currently recommended by the WHO for poliovirus surveillance.

With grab sampling, at least one-litre (1L) sample of wastewater is collected at sampling site on the date and time established for collection and specified in the site schedule, in accordance with biosafety measures outlined in **Annex 4**. From the recommended volume one litre or more (≥1L), 500 mL will usually be concentrated into ~10 mL (i.e., 50 to 100-fold concentration) in the laboratory using the polyethylene glycol/dextran two-phase separation method. The laboratory methods for concentrating the sample are described elsewhere.[1]

One litre or more of sewage is needed for the grab method of sample collection.

To be able to quantify the amount of poliovirus in environmental samples, it is important to use reproducible operating procedures for the collection and concentration of sample.

- The recommended method aims at analyzing at least 100 mL of the raw sample for virus isolation.
- The larger the volume of sewage analysed, the higher the theoretical sensitivity to detect poliovirus. However, larger volumes require handling as separate samples, thereby increasing laboratory time and workload.
- To collect **composite samples** from several sampling points (no more than 3; 2 being optimal) that serve subsegments of a larger catchment population, collectors should collect a sample of similar volume from all sampling points included in that specific site, transport them to the laboratory for mixing and creation of a final composite sample that will be concentrated and tested. *Another option* is to pre-mix the collected samples in the field (same volume for all samples) to create a single composite sample, and then transport the final sample to the laboratory for testing. For each scenario, collectors and supervisors should ensure appropriate logistics and proper labeling to avoid confusion. Mathematical modeling, however, has not found that time-composite methods were better than single time-point samples for sampling from a convergent complex network serving a large population. Furthermore, composite sample collection is tedious, resource-intensive and difficult to guarantee adherence to the schedule.
- Some sewage treatment plants have automated equipment for collecting samples at regular intervals during a 24-period time or during peak flow hours, though this equipment may be expensive and not feasible in open wastewater routes.

An example of standard operating procedures for collecting samples using the grab method are described in **Annex 5**, with forms to support sample collection in **Annex 4**.

Collecting samples via a bag-mediated filtration system (BMFS) is an alternative method accepted by the WHO and used by several countries.[21] The methodology is summarized in **Annex 7**.

2.1 – Collecting samples

To implement sample collection, the country programme should ensure field personnel are trained, equipped and supervised in a supportive environment.

- **Training:** All staff designated to collect samples need to be trained on the procedures. A sample collector and a backup are usually trained per site to ensure continuity of sample collection even when the main sample collector is not available. See **Annex 4** for guidance for sample collectors, including a detailed outline of steps in the collection process.

- **Equipment:** Collectors should have access to all supplies needed for collection, listed in **Annex 4**. Prior to going to a collection site, it is the responsibility of collectors to ensure supplies are readily available, including cold chain materials. Environmental samples must be placed in cold chain immediately upon collection, and reverse cold chain must be maintained at all times until arrival to the laboratory.
- **Supervision:** While each region should determine how often a supervisor should be present at sample collections, generally a supervisor should be present for at least 80% of all collections to ensure adherence to standard operating procedures and the sample collection schedule, to assist in filling out forms, and to help troubleshoot problems as they may occur with the site or wastewaters.

Image 4. Sample collection process.
Photo courtesy of the Global Polio Eradication Initiative image library.

During sample collection, collectors need to be aware of guidance regarding sample location, midstream sampling, and environmental conditions that may impact sampling.

- **Sampling location:** Collect sample at the "sampling point" decided during the site's initial field assessment. If there are changes in accessibility (i.e., lower or higher wastewater flow), use the following guidance to determine whether programme review is needed.
    - Within a few metres from the initial sampling point, the change is acceptable only if there are also no changes to convergent branches (i.e., neither additional nor lost branches in the catchment population).
    - With more than 50 metres from the initial sampling point, or less if the change also involves a loss or gain of convergent branches in the catchment population, the collector must consult with the supervisor and surveillance focal person before making the change. These more drastic changes in sampling point may be required because of construction or the appearance of toxicity. Once the change is approved, notification should be made in the database. (Note, if this change results in the need to open a new site, this must be discussed and agreed upon per guidance for opening a new site.)

- **Midstream sampling:** Collect samples midstream. Depending on the width and depth of the canal, sewage inlet or manhole, the collector may need to use a rope attached to a bucket or a long handle attached to a collection recipient (see cover photo).
    - Avoid the bottom of the canal where a large amount of solid debris and potentially toxic compounds may be included in the sample.
    - Avoid places where the flow is very slow or non-existent because of debris accumulation, or inadequate time of collection (i.e., missed peak flow associated with high toilet use).

- **Environmental conditions:** The following conditions should be avoided for sample collection:
    - Generally, avoid sampling during heavy rain.
        - Delay collecting samples in heavy rain to ensure personal safety, to protect equipment and to preserve the integrity of the sample as the rain may dilute wastewaters, thereby causing enterovirus concentration to be below detection levels. Delay sampling by one or two days until heavy rain subsides. Inform the laboratory if sample arrival time will be affected.

- If heavy rain or flooding precludes collection for several days in a row, consult with the supervisor and surveillance focal person to cancel scheduled sample collection. Sample collection for the month should only be canceled if a critical situation, such as flooding, earthquake or other safety concern, prevents access for a period greater than one to two weeks.
- Light rain does not preclude the collection of samples at the scheduled time.

- In cases where the smell of the wastewater and its color or other signs suggest the presence of potentially toxic compounds at the sampling point, contact the supervisor, who will:
  - explain the abnormalities under observations in the laboratory sample collection form;
  - explore the possibility of changing the sampling point or time of collection to avoid the toxicity, if it appears to be permanent; and
  - communicate changes in sampling point (location, GPS coordinates) via the laboratory form (hard copy or electronic) for proper update in the database.

2.2 – Packaging samples

Environmental samples should be packaged to prevent contamination and to ensure infectious enteroviruses within the sample are preserved for laboratory testing. See **Annex 4** for steps in packaging samples.

- **Dedicated containers:** Environmental samples should be transported to the laboratory in dedicated, robust liquid or sample containers that are packed following the "triple packing" system for biological products or diagnostic specimens. Packing samples for shipment is usually performed or supervised by a person with an infectious substance shipment certificate. AFP specimens and environmental samples should have separate cold chain containers that are appropriately labelled.

Image 5. Sample packaging and preparation for transport.
Photo courtesy of H. Abdullahi, WHO

2.3 – Transporting samples (reverse cold chain)

From the point of collection to receipt in the laboratory, samples should be maintained and shipped so they arrive intact, without the appearance of toxicity or bacterial overgrowth and with all enteroviruses preserved for testing.

- **Rapid transport:** Transportation to the laboratory should be accomplished within three (3) days of collection.
- **Reverse cold chain:** If samples cannot be shipped to the laboratory on the same day, identify facilities with cold storage capacity to act as intermediary depots such as the national laboratory or WHO country office. Samples should be maintained in a refrigerator at 4°C (range: 2°–8°C). In cases where samples will not be shipped immediately, samples should be stored at -20°C in a freezer and shipped frozen.*(3)* Sewage samples should not be stored in the same refrigerator as clinical samples for AFP, virus isolates from AFP surveillance, or any other disease because of the high risk of contamination.

While initiating ES implementation in-country, steps should be taken to identify all necessary logistics (means, routes and couriers), with focal persons identified at each stage. The programme should budget transportation costs based upon the expected number of samples per month from each site. Furthermore, field and laboratory staff should coordinate sampling schedules to minimize transportation logistics and avoid delays in testing.

In the planning process, special permits and contracts may be required. Please discuss closely with national laboratory personnel and/or the regional GPLN laboratory coordinator regarding specifics.

- **Permits:** If laboratory capacity is not available in-country and international shipment to a selected laboratory in another country is deemed feasible, identify the process required to obtain import permits from the country and the International Air Transport Association (IATA).
- **Contracts:** Because of their large volume (1L) and higher infectious potential, environmental samples may be treated differently than AFP samples for transport. Ensure contracts with courier companies include awareness and acceptance of the transportation of sewage samples conditions (i.e., sewage samples with generally low concentration of infectious pathogens versus clinical stool samples from AFP with generally higher loads of infectious pathogens).

2.4 – Environmental surveillance supervision

Recognizing the complexity of sewage sampling and its impact on the subsequent procedures, it is of utmost importance to ensure good quality activities, from sampling to arrival at the polio laboratory. Field collections should be accompanied by trained staff (i.e., supervisors) who are essential to identifying and correcting any issues observed during the collection, packaging and transport of environmental samples. For example, it has been observed and inferred that sampling time for open drains is a key factor affecting sample quality. The ES supervisor can ensure that sample collections are performed on time. Furthermore, as the collector is typically performing the physical retrieval of the wastewater sample, the supervisor can assist in completing required documentation. As ongoing practice, national programmes should ensure and document on a quarterly basis that each sampling site has undergone on-site supervision for at least 80% of sample collections. A monitoring tool for evaluating site performance is available in **Annex 6** (see Form F3a).

3. Use of information for action

3.1 – Collecting, managing and reporting data

Effective surveillance requires a platform to collect and store relevant information, either paper-based or electronic forms and databases, along with defined procedures for sharing that information with relevant stakeholders. While each country may develop their own data operations, having a minimum set of standard variables and a standardized reporting flow is essential to an ES system capable of supporting eradication.

Data collection

Several forms or checklists are available to facilitate the identification and registration of an environmental site and to assist with the collection and sharing of sample data and results (see **Table 4**).

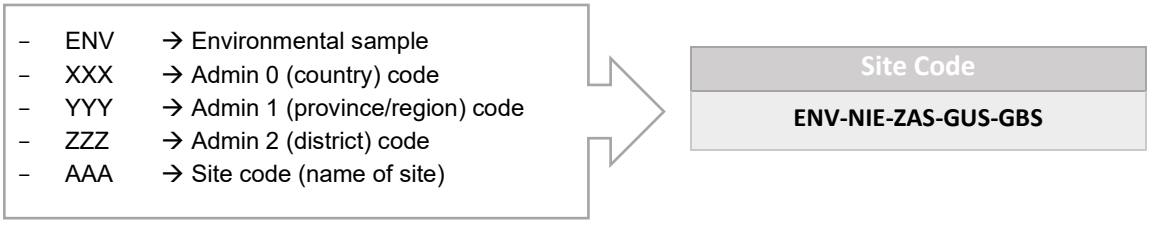
Table 4. Recommended forms

Form	*Description*
Environmental site characteristics registration *(Form F1a in Annex 6)*	Ensures core variables are collected and available to create an ES site record. Update this information annually, or when major changes are observed with the site (e.g., inactivated/closed, sampling location moved).
Digitizing a waterway *(Form F1b in Annex 6)*	Electronically collects descriptive and global positioning system (GPS) coordinates, to determine flow and accumulation of open waterways. Data are uploaded into an Environmental Site Catalogue.
Environmental sample collection *(Form F1c in Annex 6)*	Contains key information on sample collection (e.g., time of collection) and site characteristics; completed for each sample collection. In some circumstances, the *Environmental sample collection* form and *Laboratory reporting/request form* will be the same.
ES supervisor sample collection checklist *(Form F1d in Annex 6)*	Contains information on sample collection methodology and observations. Completed by the ES supervisor at the time of sample collection.

Environmental Site Characteristics Registration

The national ES focal person(s) is responsible for ensuring all core variables are available to create a site record in global data monitoring systems. The record should be created at the time the site is opened or validated and shared with data managers at the country and regional level for incorporation into national, regional and global ES databases. Core variables should be updated any time there are changes to the site (e.g., changes in sampling points that affect catchment population, changes in sampling schedule, or a site closure). At minimum, core variables for all ES sites should be reviewed on an annual basis to ensure they reflect the most up-to-date, accurate information. **Annex 6** provides a sample form to collect site characteristics and register a site (see Form F1a). The core variables for every ES site must include a site code (e.g., ENV-XXX-YYY-ZZZ-AAA) that is standardized as demonstrated in **Fig. 2**.

Fig. 2. Example of how to designate an ES site code



Following sample collection, the corresponding epidemiological identification (EPID) number will include the sample collection year and sample number. It is advisable that nomenclature differentiates environmental samples from AFP specimens.

Site characteristics for all ES sites within the country, along with their related information (such as the contact information of site collector, backup collector and site supervisor), and the polio processing laboratory should be included in the national environmental surveillance plan.

Digitizing a waterway

Catchment populations for ES sites can be difficult to quantify in settings that lack a sewer network and must therefore rely on other systems for wastewater flow, such as open canals or water channels. A set of tools have been developed to help streamline the processes to identify areas where potential candidate sites could be considered. This process incorporates GIS technology, hydrology, digital elevation models (DEM), population estimates, "blue line" data (synthetic, digital streams and waterways generated by determining flow, direction and accumulation) and the exact location and details of potential and existing environmental sites using a smartphone application with detailed GPS location. **Annex 6** provides a checklist of data needed to digitize a water (see Form F1b). Data are available for a limited number of countries in the Environmental Surveillance Site Catalogue, accessed online at: Environmental Sites.

Environmental sample collection, Laboratory reporting/request form

During each sampling visit, the collector uses this form to record pertinent information regarding sample characteristics and collection details. Programmes may opt to use separate collection and laboratory request forms or incorporate into a single data collection and reporting mechanism.

Staff may use hard-copy forms in triplicate: one copy will accompany the samples and one copy each will stay with the environmental focal person and collector or supervisor. Where applicable, bar codes to track samples may be used. If the programme uses an electronic form, such as open data kit (ODK) software for cell phones, the data should be made available to the focal person and laboratory staff. (See Form F1b in **Annex 6**.)

Testing results may be recorded on the form by laboratory staff following processing and returned to the polio programme (including the supervisor) and/or sample collectors as documentation. Furthermore, laboratory staff will usually enter this information into a laboratory database and share data with focal environmental surveillance person electronically via email or web server, per standard operating procedures. For variables that should be included in the database, see Form F2a in **Annex 6**.

Environmental surveillance supervisor sample collection checklist

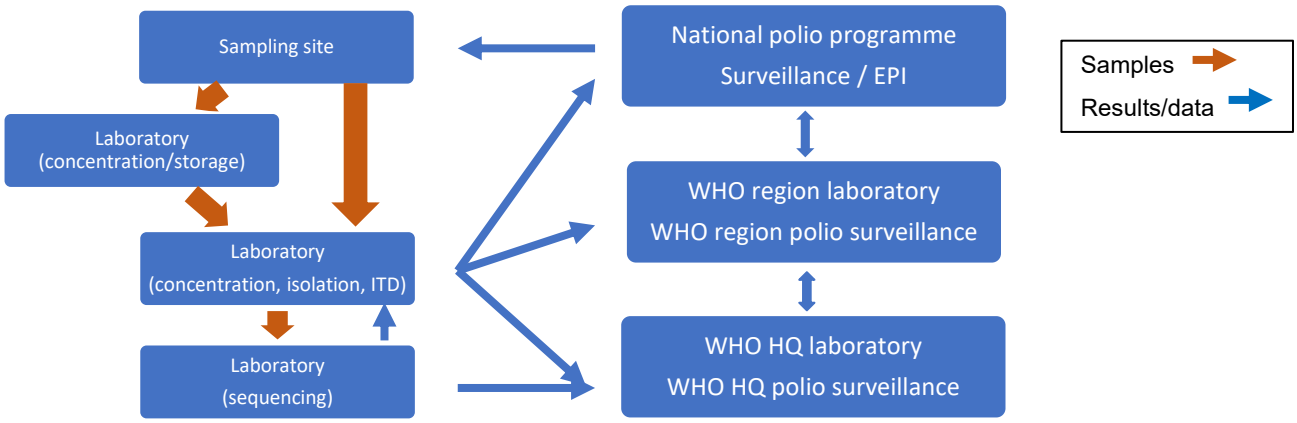
This form contains information pertinent to sample characteristics and observed collection methodology, to be filled out by the supervisor during each sampling visit. The staff may use hard-copy forms in triplicate: one copy will accompany the samples and one copy each will stay with the environmental focal person and collector or supervisor. If the programme uses an electronic form, such as ODK software for cell phones, the data should be made available to the focal person and laboratory staff. (See Form F1c in **Annex 6**.)

Information flow

The results of environmental sample testing should be reported by the laboratory and uploaded to the database immediately upon results. The WHO-accredited laboratory should ensure results are shared with the national programme in a timely and comprehensive manner and provide support for the interpretation of laboratory findings and their significance. The recommended flow of information should follow the standard system shown on **Fig. 3**. For each country ES implementation plan, the following should be

specified: (1) persons responsible for filling each form; (2) persons responsible for reporting data, sending hard-copy or electronic forms at each level; and (3) timelines for sharing forms and reporting results.

Fig. 3. Flow of samples and reporting results



EPI= Expanded Programme on Immunization; HQ =headquarters; ITD =intratypic differentiation; WHO =World Health Organization

Note: Because environmental samples often have non-poliovirus enterovirus or poliovirus enterovirus isolated, and mixtures of poliovirus require extra steps for typing and sequencing, the final laboratory results for ES samples often take longer than for AFP stool specimens.

Data management

Effective data management begins with clear data collection, transfer and storage. To date, there are several platforms available for:

- data collection (hard-copy forms, electronic forms);
- data entry (MS Access databases, Excel templates, direct entry into ODK); and
- information sharing on ES for poliovirus, allowing data to be available at the global level through the Polio Information System (POLIS).

Countries are encouraged to work with WHO regional offices to adopt a fully electronic data collection and reporting system that includes standardized variables used in national, regional and global databases.

Routine queries to update and address missing or inconsistent data should be conducted and are detailed in the next section.

3.2 – Monitoring and evaluating performance

Monitoring indicators

In the past few years, tremendous efforts have been made by countries and regions to implement the *Polio Environmental Surveillance Expansion Plan*, or PESEP.*(11)* The primary outcome of this globally coordinated effort is that hundreds of sampling sites have been opened and operationalized in countries eligible for ES implementation. Despite these gains, the previous lack of process guidance has affected the quality and consistency of some sites. The following basic monitoring framework is proposed to improve the system. Generally, in a country that is already implementing ES, addressing poor performance of existing sites

should be accorded higher priority than expanding to other areas unless there are other overriding or cogent reasons.

At the site level

Indicators to monitor ES performance at the site (and/or national, as appropriate) level are shown in **Table 5**. In environmental sites with poor performance indicators, the focal surveillance person should conduct field assessments, with support from the WHO regional office as needed.

Table 5. Performance indicators

Indicator	Calculation (expressed as a percentage)	Target	Comments
Enterovirus detection	# samples where EV (PV or NPEV) was detected / # of samples	>=50%	Analysis to be conducted per site, for 12-month period, to account for seasonality
Completeness of sample collection	# samples collected / # samples scheduled to be collected	>=80%	Each site should have a sampling schedule (i.e., monthly, fortnightly)
Timeliness of sample collection	# of samples collected on the week assigned / # of samples collected	>=80%	Each site has a scheduled week of collection to facilitate transport and laboratory workload.
	# of samples collected at the recommended time of day / # of sample collected	>=80%	Each site has a scheduled time of collection to coincide with peak wastewater flow
Condition of ES sample	# of samples that arrive in the laboratory in good condition† / # of samples arrived in the laboratory	>=80%	Good condition = volume ≥1 L*, reverse cold chain from each site maintained, no leakage
Timeliness of ES sample shipment	# of samples that arrive at a WHO-accredited lab <=3 days of sample collection / # of samples collected	>=80%	Analysis at national level; however, subnational or site-specific analyses may be useful for identifying bottlenecks or concerns
Timeliness of reporting laboratory results	# of samples with final lab results <=35 days of collection / # of samples collected	>=80%	Program staff should be aware of laboratory turn- around times to monitor for expected ES sample results
Timeliness of reporting PV laboratory results	# PV samples with sequencing results available <=7 days of receipt at a WHO-accredited sequencing lab / # of PV samples positive by ITD requiring sequencing	>=80%	

* volume specific to grab sampling method; refer to guidance related to bag-mediated filtration for appropriate volume.
Surveillance standards for vaccine-preventable diseases, second edition. Geneva: World Health Organization; 2018.
EV= enterovirus; PV= poliovirus; NPEV= non-polio enterovirus; ITD= intratypic differentiation

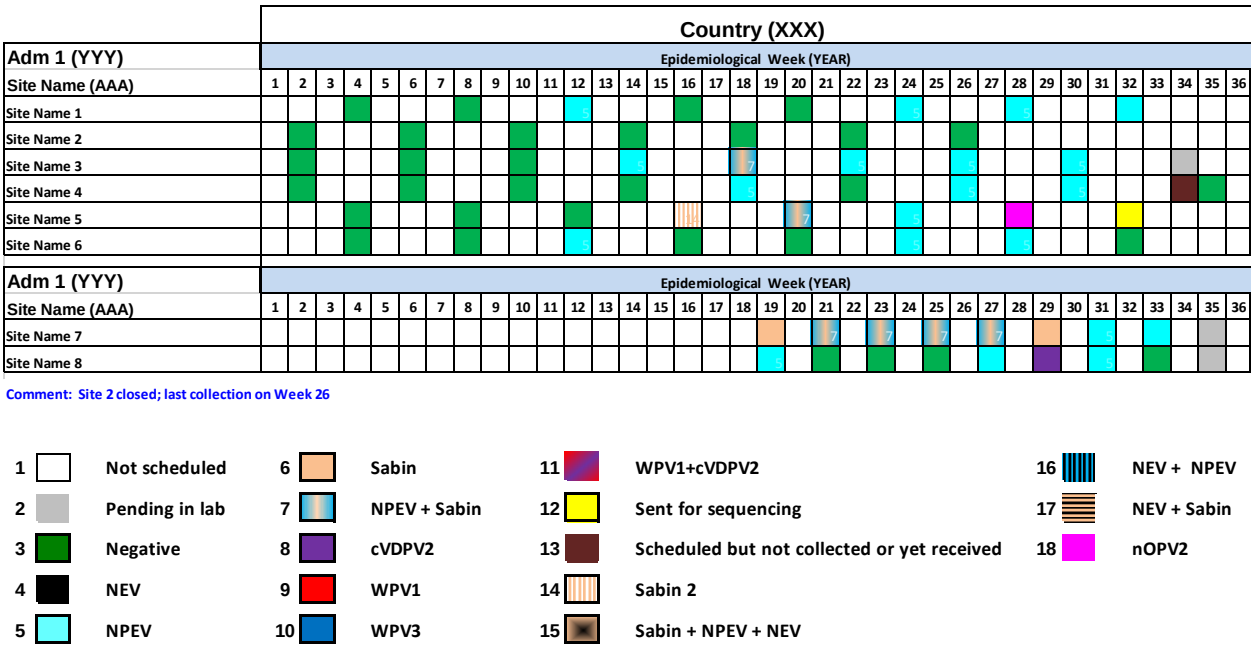
Based on observations and performance indicators, the team conducting assessments of environmental sites might recommend several actions:

- Recommend correction of procedures, keeping the sampling point in the same location/area.
- Change the location of the sampling point without affecting the general catchment population.
- Observe the site for an additional six-month period to further assess implementation.
- Close the site. A new site might be opened in a different area of the same town, if feasible, and better performance may be expected in the new site.

A checklist for conducting a field assessment systematically is provided in **Annex 6**. Electronic data collection is advisable, as information can be entered on-site and immediately uploaded to the ES data management network. In the African region, for example, checklists are available on the ODK application. Sharing the results of the field assessments with focal surveillance and EPI staff at the national, regional and global levels is crucial for monitoring data for action.

Visual assessments of site performance can be done remotely with the ES dashboard produced from laboratory data. Such visualizations are easily performed (see **Fig 4** and **Fig. 5**), and their regular review can trigger immediate action.

Fig. 4. Environmental surveillance dashboard, example



At the global and regional levels

In addition to site performance indicators and field assessments, ES monitoring at the global and regional levels may include an estimation of the footprint and sensitivity of the ES network in a country. Such an assessment is performed by detailing:

- the total number of sites per country and per province;
- the population covered by environmental sites (i.e., catchment population) as a proportion of the total population; and
- the proportion or description of high-risk populations/areas covered by ES sites.

Periodic evaluation of environmental sites and network

The performance of a country's ES system should be evaluated periodically and, when possible, integrated with AFP surveillance reviews. Regional and global WHO/GPEI staff can support analysis and field assessments. Such periodic evaluations may include the following activities:

- Conduct quarterly reviews at the subnational or subdistrict level. Coordinate with programme meetings and reviews of AFP surveillance and the EPI programme.
- Conduct desk reviews of ES at the national level every six months, to be performed by the surveillance focal staff working in coordination with regional and global WHO staff, as needed.
    - Field visits and site reassessments should be conducted for countries and sites with poor performance indicators for the last 12 months or more.
    - A comprehensive report should be shared by the national programme with WHO regional and country offices within a week of completion. It should include a calendar for scheduled on-site assessments of all sites not meeting the minimal requirement: enterovirus (EV) rate <50% and no detection of poliovirus of programmatic interest (i.e., WPV, VDPV or SL2).
- Conduct an annual assessment of ES network to include programmatic indicators, as well as any changes in the country's risk profile, broader epidemiological context or laboratory capacity as part of the country's National Environmental Surveillance Plan (**Annex 1**).
    - For all site-specific data, verify whether sites are active or inactive and confirm that all new sites have up-to-date site registration data.
        - Site disposition: a site is considered "active" if ES samples are actively being collected per the sample collection schedule, or "inactive" if ES samples are NOT being collected due to temporary or permanent suspension/closure of the site.
        - Note any changes to the site (e.g., sample collection location changed, observations of sampling site or catchment area)
    - Review the database to ensure all variables are up-to-date and accurate.
    - Once completed, the database should be shared per standard data sharing practices (i.e., feed forward to regional offices).
- External reviews (involving regional, global stakeholders) should be conducted at least every five years. Where feasible, reviews of environmental surveillance should be integrated into other polio programme reviews (e.g., AFP surveillance and immunization performance).
- Changes in the epidemiology of polio in-country and national polio eradication programme may also require in-depth assessments of the ES network that involves the addition or elimination of sites.
- At least one annual meeting including, but not limited to, all national stakeholders should be organized to present findings from assessment visits and validate a workplan for the following year.

Interpretation of data

Generally, the evaluation of the site performance and the detection of a poliovirus of interest will trigger certain recommended actions (see **Table 6**).

Table 6: Site evaluation and corrective action

Indicator monitoring (based on six months of data)	**Action required**	
	Site validation during 1st six months of opening new site	***Ongoing monitoring for existing sites***
EV isolation ≥ 50%	Validate and include site in regular ES sampling schedule. Note: a site registration form should be completed and sent to WHO regional office.	No action required.* *However, an on-site assessment should be conducted for any site with three (3) consecutive months of EV-negative results.
EV isolation <50% **and** **no PV detected (WPV, VDPV or SL)**	1. Conduct on-site assessment. 2. Evaluate all data. 3. (a) Discontinue or close the site; or (b) Start a second six-month sampling. 4. Evaluate EV rate after three (3) additional months. The decision to close a site can be made any time after six months but should not exceed 12 months.	1. Conduct on-site assessment. 2. Evaluate all data. 3. (a) Close the site; or (b) Continue to monitor over the next six-month sampling period. 4. If there is no improvement after the second six-month period, close the site.
EV isolation <50% **and** **PV detected (WPV, VDPV or SL)**	1. Conduct on-site assessment. 2. Continue collection for an additional six months. 3. Re-evaluate EV rate after three (3) months. 4. Decide to continue or close the site at 12 months.	1. Conduct on-site assessment. 2. Evaluate all data. 3. (a) Close the site; or (b) Continue to monitor over the next six-month sampling period. 4. Detection of poliovirus is optimized in sites that isolate EV in ≥ 50% of samples; decisions to keep a site open in the context of low EV detection should be periodically re-evaluated and balanced against resource availability.

EV= enterovirus; SL= Sabin-like; VDPV= vaccine-derived poliovirus; WPV= wild poliovirus

3.3 – Programme response to WPV or VDPV detection

The detection of a WPV or VDPV in the environment requires follow-up investigations to determine the significance of the findings. Various factors influence the nature and scope of the programmatic response, including:

- the status of the country as polio-free, recently endemic or endemic;
- a history of VDPV outbreaks (including in surrounding geographies);
- the level of polio immunization coverage in the population;
- the quality of AFP surveillance in the population;
- the specific goal of ES in the country; and
- the rank of the isolate (i.e., the very first or repeated observation).

Determining significance of poliovirus detection

In recently or currently polio-endemic areas, WPV or VDPV detected in the environment serves as an impetus for targeting and improving surveillance and immunization performance, especially if no concomitant paralytic cases are detected through routine AFP surveillance.

In polio-free countries, a WPV or VDPV detected through either clinical or ES strategies represent a public health emergency that warrants immediate investigation. Poliovirus detected in an environmental specimen may be derived from a single healthy person importing the virus from a non-polio-free country or region. Although it is possible to detect virus from a single person, this should be considered as extremely rare. In a well-immunized population, detection of a WPV or VDPV isolate may be determined not to pose a risk of starting transmission. However, if poliovirus is detected through ES, further laboratory and epidemiological investigations should proceed immediately to determine the significance of WPV or VDPV detected**,** to determine if there is a risk of outbreak, and to plan for any immunization response deemed necessary.

The following programmatic actions should be taken to determine if there is an outbreak:

1. *Communicate information*
   - Notify all reporting units in the country within 24 hours of receiving news of a suspected outbreak of poliomyelitis. Rapid communication regarding a possible polio outbreak is key to initiating appropriate action and preventing further spread. Request heightened active surveillance for AFP cases and strict attention to completeness and timeliness of reporting. Within 48 hours, inform the WHO that a suspected outbreak is being investigated.

2. *Enhance environmental sampling*
   - Review information on the population represented by the sampling site and the frequency of environmental sampling and determine whether there are opportunities for increasing the sensitivity of virus detection. Ongoing transmission may be deduced from repeated poliovirus detection through intensified sampling; refer to the *Standard Operating Procedures (SOPs) for Polio Environmental Surveillance Enhancement Following Investigation of a Poliovirus Event or Outbreak* for more details.*(23)*
   - Investigate additional sampling sites for surveillance of sub-populations and/or neighbouring or contact populations.

3. *Search for poliovirus-infected persons*
    - Review routine surveillance data to determine whether polio cases may have been missed. Include in the review the previous 12 months and focus on surveillance quality indicators (NPAFP detection rate, timeliness and adequacy of stool collection from cases, proportion of cases with stools tested in a WHO-accredited laboratory and the available laboratory results).
    - Review retrospective records in health facilities in the immediate and surrounding areas of the suspected outbreak to determine if polio cases were not reported or were inadequately investigated. Initiate an active case search in the suspected community.
    - Assess the value of stool surveys, taking into consideration issues related to timing, representative sampling, logistic arrangements for sample collection/handling, and assuring adequate laboratory support.

4. *Assess polio immunization coverage*
    - Review essential and supplemental polio immunization coverage to assess the likelihood of susceptible populations capable of sustaining poliovirus transmission.
    - Begin preliminary planning for an immunization response while immunization coverage is reviewed, focusing on logistic, operational and financial needs.
    - Account for the type of vaccine used (OPV, IPV or both).

5. *Enhance virologic investigations*
    - Expedite genome characterization of the WPV or VDPV isolates to assist in the investigation of their possible source and possible chains of transmission.
    - "Flag" all subsequent poliovirus isolates, environmental samples and faecal specimens from the area of the suspected outbreak for high-priority testing in a WHO-accredited laboratory.

Responding to a confirmed outbreak of WPV or VDPV

A decision should be made as soon as possible as to whether a suspected outbreak has been confirmed or if there is a sufficiently high index of suspicion to warrant an immunization response. If an outbreak is confirmed, countries should notify the WHO within 24 hours and the existing immunization services or a special steering group of experts within the Ministry of Health should advise and coordinate response activities nationwide. The response should be appropriate to the outbreak and consistent with current WHO guidelines on outbreak response. *(24)*

Annexes

Annex 1. Template for a national environmental surveillance plan

The following outline offers a template to support development of a national environmental surveillance plan.

- Background and context for ES in the country
    - AFP surveillance situation
    - Risk assessment (e.g., population movement, high-risk populations, previous outbreaks)
    - Other relevant country context (e.g., conflict, inaccessibility, immunization coverage)
    - Perceived challenges and enabling factors
- Environmental network description
    - Summary of all ES sites in-country (see the tables and figure below as examples)
    - Site-specific details (narrative, tables)
        - Rationale for site selection
            - Permanent vs. ad hoc
            - Routine, outbreak, seasonal
        - Location (administrative levels: province/region, district, city, neighbourhood)
            - Point of collection identified (description, photos)
            - GPS coordinates
        - Type of system: open, closed
        - Population size of catchment area
        - Demographics likely to be represented
        - Schedule of sample collection (note: this may be consistent for all sites or it may vary by site)
            - Recommended time of collection
            - Frequency of collection (date and schedule)
        - Other special considerations or information
    - In some circumstances, potential ES sites may not be active, but have been identified for rapid expansion, if needed. These may be noted and revisited at a later date.

Table A1 – Example national-level summary of environmental sites

S/N	Name of site	Site code	Latitude	Longitude	Province/county
1	Site 1	AAA			
2	Site 2	BBB			
3	Site 3	CCC			
4	…	…			

Figure A1 – Example map for ES site locations in-country

Note: A neighbourhood map can be a useful visual aid for the exact location of site/sample collection points.

Table A2 – Example national-level sample collection schedule

Month	Date of first collection	Date of second collection
January	15th January	29nd January
February	12th February	26th February
March	12th March	26th March
April	9th April	23rd April
May	7th May	21st May
June	4th June	18th June
July	2nd July	16th July
August	6th August	20th August
Sept	3rd September	17th September
October	1st October	15th October
November	5th November	19th November
December	3rd December	17th December

- Roles and responsibilities
    - Country ES focal point(s): Name, contact information (including organization: WHO, MOH)
    - Field personnel
        - Primary and back-up sample collectors (name, contact information)
        - Supervisor (name, contact information)
        - Contact at site (other personnel)
    - Laboratory focal person

    - Other stakeholders (e.g., local traditional leaders engaged for community buy-in; security)
- Standardized protocols for field activities
    - Sample collection (e.g., grab), transportation, supervision SOPs
    - Laboratory request form for sample submission (see Form F2a in Annex 6)
    - Other forms (as needed)
- Standardized protocols for laboratory*(1,4)*
    - Laboratory protocols including method of concentration (e.g., two-phase, BMFS)
    - Primary lab-identified transportation/shipping SOPs
    - Provision of adequate laboratory space (to which lab will samples be sent for processing)
    - Personnel
    - Equipment and reagents; procurement
    - Contingency plan
- Logistics
    - Provision of transport of samples (from collection point to lab); storage (as needed) for samples
    - Transportation needs; vehicles, travel arrangements, security
    - Contingency planning
- Data management and reporting systems
    - Data collection (electronic, hard copy); if electronic, system used (e.g., ODK)
    - Data reporting (including content of report and reporting channels)
        - Schedule of data reporting to regional office (e.g., weekly)
        - Inclusion of ES data in surveillance updates and other feedback platforms in country; polio situation report (SITREP), if applicable
    - Analysis and feedback of results to stakeholders (WHO, MOH, field personnel)
        - Frequency and format (i.e., laboratory dashboard, reports, verbal)
- Training and quality assurance
    - Onboarding of new sample collector
    - Schedule for regular ES training
    - Schedule of regular meetings (field and lab)
    - Feedback of ES site performance and documentation of action taken
- Programmatic response to both positive and negative laboratory findings
    - Monitoring plan (national level; external review)
    - Data monitoring (field and lab)
    - Communication plans
- Finance
    - ES field and laboratory components should be differentiated in surveillance budgets
    - Funding source and requisition information

Annex 2. Sample agenda for an in-country initiation mission

In general, the WHO country office will facilitate the in-country ES initiation mission.

Please note, the following sample agenda does not factor in travel time to visit sites, number of sites under consideration, pre-mission preparatory work or potential external conflicts (e.g., scheduling conflicts or weather delays). Furthermore, discussions of financing (to include cost and source of funding) should be conducted to ensure adequate funding is available for ES.

Table B1. Example agenda for an in-country initiation mission

Day/date	*Description*
Day 1	Briefings at national level, including WHO country office (CO, MOH, city council, laboratorians, etc.)
	Training or briefings on terms of reference for the visit
Days 2 and 3	Site selection, observation and data collection (e.g., digitize waterways), and prioritization
Day 4	Training (theory and application session), including early morning visit to sites (e.g., 5–9am) to observe best flow rate Ensure appropriate field materials (forms, sample collection, and personal protective equipment) are available Download and practice with electronic forms
Days 5 and 6	Collection of first sewage samples to be sent to the laboratory
	Develop, refine and finalize the National Environmental Surveillance Plan
	Briefing on data management, including monitoring and reporting on ES performance
	Share ES initiation report with GPLN (laboratory identified to test samples). Include key information on: – List of sites – Sewage sample collection schedule – Names and contact information of collectors and supervisors by site – Address of surveillance/EPI focal point (for correspondence if laboratory has issues)
	Discussion of data sharing (e.g., laboratory shares weekly data with the CO surveillance focal point)
Day 7	Outbriefings to include summary of activities conducted and way forward

Annex 3. Rapid tool to assess urban areas

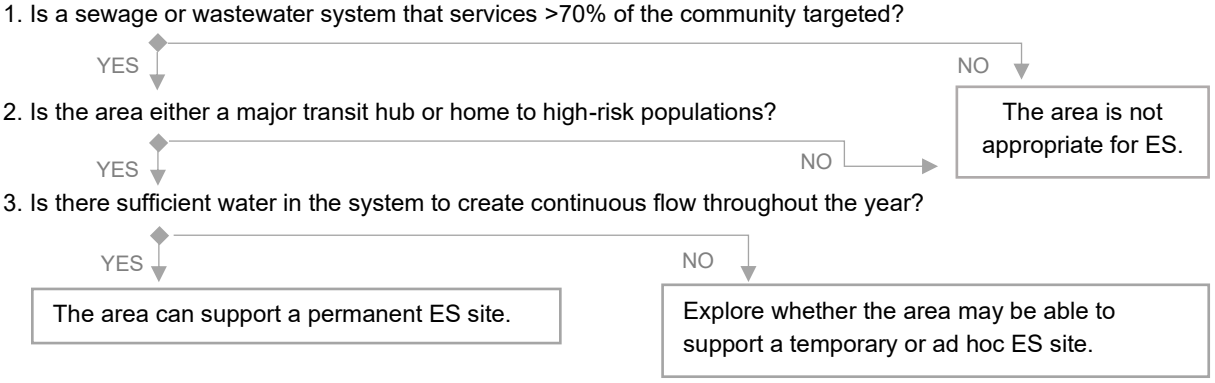
The following tool may be used by polio surveillance, ministries of health or other public health stakeholders to rapidly assess if an urban area can support environmental surveillance.

While a rapid assessment does not necessarily indicate that ES can or will be initiated, results of such assessment will provide useful background documentation for further discussions with ES subject matter experts (i.e., regional ES working groups, GPLN laboratory personnel).

Figure C1. Rapid tool for assessing ES in urban areas

Step one: List all cities or urban areas at risk for involvement in an outbreak with: (1) a total population ≥ 500,000, or (2) a population between 100,000–500,000 and lie within 10km of a major international border crossing.

Step two: With local officials, answer the following questions:



Annex 4. Biosafety measures to reduce field worker health risks

Essential training

- All workers who handle human waste or sewage should receive training on disease prevention.
- The training should include:
    - information on basic hygiene practices;
    - use and disposal of personal protective equipment (PPE);
    - proper handling of human waste or sewage; and
    - promptly seeking medical attention when sick.

Basic hygiene practices for field workers

- Wash hands immediately after handling human waste or sewage.
- Avoid touching body parts while handling human waste/sewage.
- Keep open sores/cuts/wounds covered with clean dry bandages.
- Flush eyes with clean water if exposed to human waste/sewage.
- Use gloves to prevent exposure of waste/sewerage to open skin.
- Wear rubber boots at the collection site of waste/sewage.
- Remove rubber boots and PPE before leaving collection site.
- Clean contaminated work clothing with 0.05% chlorine solution.
- Safely dispose of all wastes generated during collection of waste/sewage.

Personal protective equipment

- Wash hands with soap and clean water *immediately after* removing PPE.
- The following PPE is recommended:
    - goggles to protect eyes from splashes of human waste or sewage;
    - protective face mask or splash-proof face shield to protect nose and mouth from splashes of human waste or sewage;
    - liquid-repellent overalls to keep human waste or sewage off clothing;
    - waterproof gloves to prevent exposure to waste or sewage;
    - rubber boots for sample collector to prevent exposure to human waste or sewage; and
    - water-proof disposable shoe covers for supervisor/assistant.

Vaccination recommendations for workers

- Develop vaccination recommendation in consultation with local health authorities.
- Vaccination recommendations might include:
    - polio
    - tetanus
    - typhoid fever
    - cholera
    - hepatitis A
    - hepatitis B

Annex 5. Standard operating procedures for wastewater sample collection (grab method)

Responsible staff

- Collector of environmental samples
- Supervisor of environmental sample collection

Supplies

Reusable	*Disposable*
✓ 5-litre bucket ✓ Robust liquid container (1-1.5 litres) *aka "jerry can"* ✓ Rope or stick (~7 metre) ✓ Plastic funnel ✓ Rubber boots and thick rubber boots ✓ Permanent marker ✓ Pen ✓ Phone with ODK software installed (as relevant) ✓ Dedicated vaccine carrier (marked for transport of environmental samples only)	✓ PPE: surgical mask or respirator, disposable gloves, gown or apron ✓ Liquid bleach, water and gauze or paper towels to clean supplies ✓ Parafilm tape ✓ Prefilled labels (with bar code, if available) ✓ Plastic bags or small zippered bags for paper forms, large bags for samples ✓ Frozen ice packs inside the dedicated vaccine carrier (both ice packs and vaccine carrier should be used for ES only)

Collection of wastewater samples

1. The day before the scheduled sample collection date, the collector/supervisor will collect the supplies, arrange for the availability of frozen ice packs and plan transportation to the sampling site and laboratory.
2. On the day of collection, the collector and supervisor will arrive at the sampling point at the time scheduled for collection in that sampling point. If there is heavy rain, the collector will consult with the supervisor to delay collection by a day or two.
3. Once at the sampling point, the collector will don PPE, including mask, apron and gloves.
4. The collector will use the bucket and rope to reach into midstream of the canal/sewage inlet and collect a sample of wastewater.
5. Using the funnel, the collector will transfer at least 1 litre of wastewater to a robust sample container.
6. The collector will close the container tightly and seal the top with parafilm tape to prevent leakage and then clean the surface of the container with liquid bleach and let it dry.
7. The collector/supervisor will write on the container using a permanent marker or use a label or with the following information:
   a) EPID number for the sample site;
   b) the number of sample for the year;
   c) the date and time of collection;
   d) the name of collector; and

e) the barcode, if a label-maker is available and electronic formulary allows scanning. (If using an electronic form, the site ID number and the sample number will be pre-populated. The barcode will be scanned to facilitate sample tracking in real-time.)

8. The collector will place the labeled container inside a large plastic zippered bag, ensuring there is no leakage, and then place the sample inside the cold box / sample carrier.
9. Before leaving the site, the collector will wash the bucket and funnel with liquid bleach, rinse with water (if available) and let them dry for next use. The collector will dispose of used gloves and clean hands with water and soap, alcohol or sanitizing towels.
10. The supervisor/collector will complete the laboratory request form (see Form F2a in Annex 7).
    - The hard-copy form is placed inside a zippered plastic bag into the carrier with the sample.
    - The electronic copy will be filled out by the supervisor on a cellphone using ODK software. The ODK form will require GPS coordinates and the barcode, which will facilitate laboratory tracking of the sample in real time.

Transportation and storage of wastewater samples

1. The collector/supervisor will transport the sample carrier to the laboratory or depot facility for storage at 4–8°C for up to three (3) days, or frozen if more time is required until shipment to the laboratory can be arranged. The collector/supervisor will share the hard copy of the laboratory form with the person in charge of samples storage and shipment.
2. If samples are kept in a refrigerator, it should be one that is dedicated to ES (i.e., separate from AFP specimens).
3. Samples should arrive at the polio laboratory with the laboratory request form within three (3) to seven (7) days of collection, depending on the location of polio laboratory.
4. The laboratory should be notified in advance and it should acknowledge the receipt of the samples.

Important to remember:

- **Frequency of collection**: Follow the collection schedule (e.g., one or two samples per month).
- **Time of collection**: Collect samples at the recommended time of the day (e.g., early morning before 07:00 am).
- **Equipment:** Use designated equipment only and use PPE.
- **Reverse cold chain:** Use recommended number of ice packs (e.g., ≥5) and transport immediately after collection.
- **Heavy rain:** Postpone collection until the following day.
- **Monitoring:** Ensure that the sample is taken by **personnel who have been trained in sample collection** and under the **supervision** of the focal point.

Annex 6. Forms, tools and checklists

1. Data collection resources

Form F1a. Environmental site characteristics registration form

- **Site name**: short description usually referencing the place, street, or landmark
- **Site EPID number**
- Site category for surveillance role: permanent / temporary
- Reason for opening site: (open text)
- Geographical information:
    - **Location: Country, state/province, district** (Admin 0, Admin 1, Admin 2)
    - **GPS coordinates of sampling sites**: X coordinate, Y coordinate
    - Map of draining waterways
- **Composite site (yes or no)**
- Features of the sewage system:
    - Open/closed/other
    - Flow: annual, seasonal
- Sampling schedule:
    - **Date when sampling started (i.e., site opened)**
    - Collection frequency (weekly, fortnightly, monthly)
    - Scheduled week-year and/or day of week (i.e., Monday)
    - Scheduled time (or range) for sample collection (i.e., 06:30)
    - **Date when sampling stopped in this site (i.e., site closed)**
- Estimated size and type of catchment population
    - Estimated total catchment population
    - Catchment geographical area (street boundaries)
    - Type of risk-population included
- Focal person responsible for entering and updating information (e.g., National ES focal person)

Addendum for changes in site

- Date of change
- Person responsible for change
- Update: (open text explaining the change)
  The new data will also be updated in the database. For example, "replaced sampling point because monitoring demonstrated poor performance."
- New GPS coordinates (new catchment area, if changed; new population size, if changed)
- Disposition: a site is "active" if ES samples are actively being collected per the sample collection schedule, or "inactive" if ES samples are NOT being collected due to temporary or permanent suspension/closure of the site.

*__Core variables – should be shared with RO and HQ for all new and existing sites;__ required elements for creating a site record in POLIS

Form F1b. Checklist for digitizing a waterway

- Country
- Province
- District
- Site code
- Site name
- Waterway name
- Waterway description
- Waterway type
- Formal/concrete ditch, informal (dirt), natural (e.g., river)
- Flow type (annual, seasonal)
- Comments
- GPS coordinates for each waterway point
- Picture
- Waterway point details
    - Width (units)
    - Depth
    - Debris assessment (little/none, moderate, heavy)
    - Junction type (none, sewer, waterway, factory dump)
    - Comments

Form F1c. Environmental sample collection

- Name of the sample collector
- Location
    - Country
    - State/province
    - District
    - Ward/health area *(describe)*
- Site identification/characteristics
    - Name of collection site (pre-populate or have list of site names)
    - Site EPID number (site collection number)
    - Barcode (if available)
    - Type of site (type of sewage plant or sewage system)
- Date and time of sample collection
- Observations during collection
    - Time of collection
    - Was there good flow rate at the time of collection?
    - Was the sample collected at the designated point at the site/location?
    - Was the sample amount/volume collected adequate?
    - Are there any industries or facilities discharging chemical effluents at the time of sample collection?
- Date sample sent to laboratory
- Intermediary laboratory / health facility / WHO or MOH office
    - Date sample was received in the intermediary laboratory
    - Person receiving sample (name, designation, signature)
    - Person shipping sample to the processing laboratory (name, designation, signature)
    - Date sample was shipped to the processing laboratory
- Note any comments or observations during collection (open text, blank if nothing relevant). Mention issues such as collection delayed because of rain, description of wastewater color or smell that could be associated with presence of toxic compounds, changes in sampling points because access blocked by construction or other). State whether there was any need to store samples before shipment.

Note: In some instances, the form for environmental sample collection and the laboratory reporting/request form (see Form F2a below) will be the same.

Form F1d. Supervisor sample collection assessment checklist

- Name of the supervisor
- Location
    - Country
    - State/province
    - District
    - Ward/health area *(describe)*
- Site identification/characteristics
    - Name of collection site (pre-populate or have list of site names)
    - Site EPID number (site collection number)
    - Barcode (if available)
    - Type of site (type of sewage plant or sewage system)
- Date and time of sample collection
- Sample collector name
- Observations during collection
    - Time of collection
    - Was there good flow rate at the time of collection
    - Was the sample collected at the designated point at the site/location?
    - Was the sample amount/volume collected adequate?
    - Are there any industries or facilities discharging chemical effluents at the time of sample collection?
    - Was the information on the sample container and the data tool correctly filled?
    - Were barcodes for tracking the ES samples used as recommended?
    - Did the sample collector use appropriate personal protective equipment?
        - If not, was it missing?
- Did the sample collector receive allowance on time?
- Date sample sent to laboratory
- Intermediary laboratory / health facility / WHO or MOH office
    - Date sample was received in the intermediary laboratory
    - Person receiving sample (name, designation, signature)
    - Person shipping sample to the processing laboratory (name, designation, signature)
    - Date sample was shipped to the processing laboratory
- Was there any time during the last three (3) months when the sample collection was not done according to the schedule?
    - Note any comments or observations during collection (open text, blank if nothing relevant). Mention issues or deviations to collection; e.g., such as collection delays due to heavy rain, description of wastewater color or smell that could be associated with presence of toxic compounds, changes in sampling points due to access issues). State whether there was any need to store samples before shipment.

2. Laboratory resources

Form F2a Laboratory request/report form

- Country, country code
- Sample number
- ID code: EPID (e.g., ENV-XXX-YYY-ZZZ-YY-###)
- Sample collection information
    - Location
        - State/province (province code)
        - District (district code)
        - Sub-district/ward/health area/neighborhood *(describe)*
        - Location (other)
    - Site identification/characteristics
        - Name of collection site (pre-populate or have list of site names)
        - Site code
        - Geo-coordinates of site (latitude and longitude)
        - Barcode (if available)
        - Type of site (open, closed)
    - Date of sample collection
    - Time of sample collection
    - Date sample sent to laboratory
    - Name of person who collected sample (telephone, signatures)
    - Name of supervisor during collection (telephone, signature)
- Laboratory
    - Date sample received at laboratory
    - Name of person receiving sample at laboratory (signature)
    - Sample lab ID number
    - Condition of sample at receipt (good, poor)
    - If poor, specify details related to sample quality
    - Temperature of carrier on arrival in lab (C)
    - Volume of specimen (litres)
    - Color of specimen (clear, cloudy, dark)
- Results
    - Final cell culture results
    - ITD results
    - Sequencing results
    - Date results sent out by lab
    - Date results received by surveillance (or WHO)

3. Monitoring resources

Form F3a. Monitoring checklist

- Interviewer name
- Date of assessment
- Country
- Participants in the assessment (include role, agency, length of time in position, date of most recent polio surveillance training)
- Documentation and coordination
    - Availability of ES documents (e.g., National Environmental Surveillance Plan, standard operating procedures, external review reports)
    - Document validated
    - Last updates
    - Agencies / local authorities involved in selection of site
- General information about the ES network
    - Total number of operational (active) sites in the country (routine, adhoc)
    - Availability of blue lines for sites
    - Geo-coordinates for all sites
    - Annual v. seasonal flow
    - Open v. close type sewerage system
    - Catchment population description (inclusion of high-risk populations, drainage from socio-economic facilities [hospital, schools, markets, factories, etc.])
    - Has optimum sampling location and time of collection been determined for all sites?
    - Last OPV containing SIA (location, date)
    - Frequency of collection (availability of schedule)
    - Contact list of all supervisors, collectors and backups for each site
    - Frequency of supervision of sites
- Resources and logistics
    - Collectors received transport allowance in last three (3) months
    - Availability of reserve stock of PPE and other supplies for collectors at national level. Note which supplies are missing or needed.

☐ Bucket (collection) ☐ Funnel ☐ Sample transport container
☐ Disinfectant/ liquid bleach ☐ Rope/extensor ☐ Transport packing materials
☐ Towels for drying ☐ Labels ☐ Ice packs
☐ Permanent markers ☐ Blank laboratory forms
☐ Personal Protective Equipment (gloves, gown, etc.)

- Laboratory support system
    - Laboratory for ES processing
    - Available storage facilities (national, sub-national) until shipment to lab
    - Storage of ES samples (e.g., separate refrigerator than AFP specimens)
    - Length of time for sample storage before shipment (average, longest)
    - Percent of samples reaching lab in good condition

    - Processing lab (if applicable):
        - Availability of staff to process ES samples
        - Frequency of data sharing
        - Supply or reagent shortages; frequency of inventory checks
        - Any testing/processing delays
- Performance monitoring
    - Total number of sample collections expected during (6 or 12 months)
    - Proportion of collections completed
    - Proportion of collections done on time (per schedule)
    - Proportion of collections supervised (use of ODK or electronic tool)
    - Enterovirus isolation rate
- Summary of findings and recommendations

Annex 7. Other methods of sewage sample collection

Bag-mediated filtration system

The bag-mediated filtration system (BMFS) involves sampling and initial concentration of sewage water at the sample collection site using simple equipment. The process of collection and filtration may require 60 to 90 minutes at the environmental site, depending on the amount of solids in the water. If filtration cannot be done on the environmental site for security reasons, the collection bag may be transported to the laboratory or alternate site in cold chain using a Kool Bucket.™

The basic BMFS equipment for sewage sample collection consists of a 6- to 12-litre collection bag, a ViroCap™ filter (Scientific methods, Inc. Granger, IN, USA), a collapsible tripod stand, tubing to conduct the filtration, PPE and decontamination supplies. The collection bag and filters may be single- or multiple-use. The collection bag includes: (1) a mesh covered opening to exclude refuse and debris, and (2) an open tubing adapter port at the bottom for discarding settled solids. The collection bag slopes toward the opening, which facilitates discarding of sediment. The ViroCap ™ filter traps adsorbs viruses using electrostatic interactions and is encased in a polycarbonate sump. To improve virus survival until elution, a preservative may be added to the filter before sending to the field.*(13)*

The collectors follow the following steps to collect an environmental sample using the BMFS.**

- After donning protective equipment, assemble the tripod on a stable surface at the collection site.
- Place a mesh over the opening of the collection bag using a metallic ring with a clamp and attach a rope to the ring. Also attach tubing at the bottom of the bag and close the tubing clamp.
- Drop the bag into the wastewater and drag it slowly until ~ 3-5L of water are collected. As an alternative, the sample can be collected with a bucket and poured into the collection bag.
- Pull the bag out of the water and hook it on a tripod stand.
- Record water volume and wait for ~15 minutes to allow solids to settle in the bottom of the bag. Next, drain the solids into a separate collection bag until a clarified liquid appears. Record the water level placing a mark on the collection bag.
- Attach the filter to the collection bag using special tubing and begin filtration. The filter should be labeled with the sample ID number, date and time of collection.
- Wait for a full 3L to pass through the filter or up to 40 minutes, whichever is longer. If the full volume is filtered, pour the solids back into the bag and continue filtration until the volume has completed or the filter gets obstructed.
- Remove the filter from the tubing, place caps to the inlet, wipe with bleach, place into a plastic bag and store in cold chain.
- Discard the leftover wastewater. Clean the tripod with bleach, and place disposable and reusable items in biohazard bags for future autoclaving or discarding, as appropriate.
- Transport the filter and materials to the laboratory

Once in the laboratory, laboratory staff must elute the sample from the filter within 48 hours using a manual or electric pump. The process produces an eluate volume of about ~100 mL, which goes through a second concentration to obtain about 10-15 mL of concentrate. This concentrate is treated with chloroform and antibiotics for future testing for poliovirus, using the standard WHO procedure.

** A step-by-step guide of BMFS procedures (with photos) has been made available online by the Environmental and Occupational Health Microbiology Laboratory of University of Washington. Accessed on 10 July 2021. (https://path.ent.box.com/s/75berzfewlxzzzfj70xue8wywb3qp2q6)

Annex 8. Examples of environmental surveillance sites

All photos courtesy of O. Diop, WHO, and H. Abdullahi, WHO.

Example of a wastewater treatment facility (closed system)

Image H1. A closed wastewater treatment facility.

Examples of open canals or drainage

Image H2a.

Image H2b.

Image H2c.

Examples of poor or sub-optimal sites

Image H3a. Site is dry.

Image H3b. Water in an open field (possible rainwater); not sewage.

Image H3c. Possible broken pipe; stagnant water with no flow; possible chemical contamination.

Image H3d. No flow; dumping site.

Image H3e. Open drainage with no flow.

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