Global Polio Eradication Initiative

# GUIDELINES ON ENVIRONMENTAL SURVEILLANCE FOR DETECTION OF POLIOVIRUSES

**Working draft - March 2015**

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## List of abbreviations used

| | |
|---|---|
| AFP | acute flaccid paralysis |
| BSL-2 | Biosafety Level 2 |
| cVDPV | circulating vaccine-derived poliovirus |
| EPI | Expanded Programme on Immunization |
| GPEI | Global Polio Eradication Initiative |
| IPV | inactivated polio vaccine |
| ITD | intratypic differentiation |
| iVDPV | vaccine-derived poliovirus from immunodeficient individuals |
| NID | National Immunization Day |
| NPEV | non-polio enterovirus |
| NPL | national polio laboratory |
| NPP | National Polio Programme |
| NSL | Non Sabin-like (vaccine virus) |
| OPV | oral polio vaccine |
| PEG | polyethylene glycol |
| PV | poliovirus |
| rRT-PCR | real-time reverse transcription polymerase chain reaction |
| SL | Sabin-like (vaccine virus) |
| SIA | Supplementary Immunization Activity |
| SOP | Standard Operating Procedure |
| tOPV | trivalent oral polio vaccine |
| VDPV | vaccine-derived poliovirus |
| WHO | World Health Organization |
| WPV | wild poliovirus |

## 1. Potential roles for environmental surveillance

There are two distinct but related roles for environmental surveillance, investigating sewage or wastewater, in support of the Global Polio Eradication Initiative (GPEI). The first is targeted directly at detecting circulating polioviruses (PV) as a supplementary method in support of disease-based acute flaccid paralysis (AFP) surveillance for suspected polio cases. Environmental surveillance can assist in identifying residual wild poliovirus (WPV) transmission in endemic areas, particularly where WPV circulates among infected individuals not showing signs of paralysis. Environmental surveillance can be a valuable tool to provide an early indication of new PV importations into polio-free areas or of vaccine-derived polioviruses (VDPV) transmission, and to confirm the presence of vaccine-related virus following a vaccination campaign using oral polio vaccine (OPV).

As the GPEI moves towards achieving the goal of polio eradication, the second major role for environmental surveillance, that of providing evidence for certification of polio-free status, will come into prominence. Environmental surveillance data may also provide valuable documentation on the disappearance of Sabin-related vaccine viruses from the environment during the period of transition from OPV to IPV use. According to the *Polio Eradication and Endgame Strategic Plan 2013-2018*[1], the target date for stopping global use of all OPV is 2019-2020, and environmental surveillance should play a significant role in monitoring and providing evidence for the absence of vaccine-related viruses following cessation of OPV use. Data generated from continued high-quality environmental surveillance in existing sampling sites will provide important evidence to be considered before the decision can be made to certify the world as polio free.

This document is intended to update previous WHO guidelines on environmental surveillance in support of the GPEI in light of experience gained and technological advances made over the past decade. In addition to updating the technical details, greater emphasis has been placed on the programmatic need to assess and evaluate the utility and appropriateness of efforts and plans for the strengthening and expansion of environmental surveillance.

[1] The *Polio Eradication & Endgame Strategic Plan 2013-2018* (WHO/POLIO/13.02). Available online: http://www.polioeradication.org/resourcelibrary/strategyandwork.aspx

## 2. Background

### *2.1 Biological basis*

Effective environmental surveillance relies upon the PV excretion pattern characteristically exhibited by infected individuals. PV transmission is person-to-person, both via the faecal-oral and the oral-oral routes (the latter being most probable in developed countries with high hygiene standards). Irrespective of the presence or absence of clinical symptoms, PV replication in infected individuals initially occurs in the pharynx and usually continues in the para-intestinal submucosal lymphatic tissue for several weeks to a few months after initial infection. Throughout this period virus is excreted into the faeces and shed into the environment. Shedding may be intermittent and is affected by the immune status and immune competence of the individual. Past natural infection with WPV and vaccination with OPV serve to significantly reduce the extent and duration of PV shedding. The amount of virus excreted into stools is known to be variable, with maximal amounts reaching $10^7$ infectious doses/day per person (1).

Poliovirus remains infectious in the environment for varying lengths of time depending on the immediate conditions. PVs are relatively stable in aqueous environments at ambient temperatures, and adsorption to various solid materials in the environment may further extend the time over which at least part of the infectivity can be recovered (2). Early studies demonstrated that increased temperature, ammonium concentrations and pH are major factors in the natural inactivation of PV in sewage. The average time required for a 90% decrease (corresponding to approximately 1 $\log_{10}$ unit) in enterovirus titre in sewage sludge has been reported as 180 days at 2$^{o}$C and 26 days at 23$^{o}$C

Factors that affect transmission of the virus include extent of crowding, levels of hygiene, water quality, and sewage handling facilities. In general in endemic countries and areas, WPVs (in contrast to the Sabin-like (SL) vaccine viruses from OPV) have a distinct seasonal pattern of circulation that varies by geographic area. In tropical and semitropical areas circulation tends to be year round, or often associated with the rainy season. Prior to poliovirus immunizations, in temperate areas polioviruses were most prevalent in the summer and fall, although outbreaks could continue into the winter. SL virus should be detectable at all times in countries with routine use of OPV or detections may cluster around the time of national or sub-national supplementary immunization activities (SIAs).

Not all PV shed in the faeces is readily available for detection through environmental surveillance. Populations living in dwellings with water toilets connected to a converging sewer network can be monitored by collecting representative samples from downstream major collectors or sewage treatment plants. Other systems used for the handling of human waste pose significant challenges for collection of representative environmental samples and may significantly decrease the value of environmental surveillance in monitoring for PVs.

### *2.2 Uses in polio surveillance*

The systematic examination of stool samples from patients identified through AFP surveillance links PV isolates directly to specific individuals and permits a detailed and focused investigation of that individual, the immediate community at risk, and the virus transmission routes involved. While AFP surveillance can, in principle, be applicable to any human population at any time, situations arise in which there are good reasons to question the reliability of negative results from AFP surveillance. The examination of composite human faecal samples through environmental surveillance links PV isolates from unknown individuals to populations served by a common wastewater collection system. While not linking PV isolates directly to infected individuals, environmental surveillance can provide valuable supplementary surveillance information, particularly in high-density urban populations where AFP surveillance is absent or of poor quality, persistent virus circulation is suspected, or

frequent virus re-introduction is observed or suspected. Semi-quantitative environmental surveillance for poliovirus during silent transmission can conserve limited resources by pinpointing those communities where stool surveillance of asymptomatic individuals is most likely to yield poliovirus positive stools to provide an evidence-based targeted intervention response (4).

Monitoring of sewage or wastewater, later referred to as environmental surveillance, has been an important component of PV surveillance for many years in several developed countries and has played an increasingly important role in disease eradication programs in selected areas of developing countries (5). Environmental surveillance has played a key role in documenting the elimination of indigenous WPV in Egypt (6, 7) and India (8, 9), and currently plays an important role in the eradication of WPV from the remaining endemic foci in Pakistan and Nigeria.

Environmental surveillance has also played a key role in the detection of reintroduction of WPV into polio-free areas (10, 11) (see ***Table 1***) and assisted in the detection and identification of both circulating vaccine-derived polioviruses (cVDPVs) and vaccine-derived polioviruses from immunodeficient individuals (iVDPVs) (see ***Table 2***). Routine systematic environmental surveillance programs for detecting poliovirus initiated in Finland in 1984 and in Israel in 1988 in response to poliovirus outbreaks during those years, have continued to date (2). Highly diverged vaccine derived polioviruses have been intermittently recovered from environmental surveillance samples by both national surveillance programs providing evidence for the presence of individuals with persistent poliovirus infections (2). In Israel, environmental surveillance was used to follow the movement between cities and within cities of individuals persistently infected with vaccine-derived poliovirus (12), to document multiple introduction of wild polioviruses between 1990 and 2013 (3, 11, 13, 14), to follow silent transmission of some of these imported wild polioviruses and the effectiveness of SIAs carried out in response to silent circulation.

| Country | City or region | Year | Serotype | Source of virus | Reference |
|---|---|---|---|---|---|
| Brazil | Sao Paolo | 2014 | 1 | imported | † |
| Afghanistan | Multiple | 2014 | 1 | indigenous, imported | † |
| Colombia | Cartagena | 1991 | 1 | Indigenous | (15) |
| Egypt | Multiple | 2000-2005 | 1 | Indigenous | (6) |
| | Cairo | 2008 | 1 | Imported | (16)(17) |
| | Giza | 2008 | 1 | Imported | (16) (17) |
| | Aswan | 2010 | 1 | Imported | (17) |
| | Cairo | 2012 | 1 | Imported | (10) |
| India | Mumbai | 2001-2010 | 1,3 | Indigenous | (5) (18) |
| | Delhi | 2010 | 1,3 | Indigenous | † |
| Israel, West Bank and Gaza | Gaza | 1991-2002 | 1 | Imported | (19) (14) |
| | Tel Aviv | 2013-2014 | 1 | Imported | (11) † |
| The Netherlands | Multiple | 1992-1993 | 3 | Imported | (20) |
| Kenya | Nairobi | 2013 | 1 | Imported | † |
| Nigeria | Multiple | 2012-2013 | 1 | indigenous | † |
| | Kano, Lagos | 2012 | 3 | indigenous | † |
| | Kaduna | 2014 | 1 | indigenous | † |
| Pakistan | Karachi | 2009-2010 | 1,3 | Indigenous | † |

| | | | | | |
|---|---|---|---|---|---|
| | Lahore | 2009-2014 | 1 | indigenous | † |
| | Multiple | 2010-2014 | 1 | indigenous | (21)† |
| Switzerland | Geneva | 2007 | 1 | Imported | (22) |

† Routine notification to WHO.

**Table 1. Global detection of WPV from environmental samples – 1991 to 2014.**

| Country | City or region | Year | Serotype | Reference |
|---|---|---|---|---|
| China | Shandong | 2012-2013 | 2 | (23) |
| | Guangdong | | | |
| Egypt | Multiple | 2004-2009 | 2 | (17) (24) |
| | Cairo | 2010 | 1 | (17) |
| | Multiple | 2012-2013 | 2 | (23) |
| | Multiple | 2012-2014 | 1 | (23)† |
| | Giza | 2014 | 2 | † |
| Estonia | Tallinn | 2002 | 3 | (25) |
| | | 2008, 2009 | 2,3 | (26)(27) |
| | | 2010 | 2 | (26) |
| | | 2012 | 2 | †∞ |
| Finland | Tampere | 2008 | 1, 2 | (28) |
| | | 2009-2010 | 1, 2, 3 | (28) (27) |
| | | 2011 | 2 | †∞ |
| | | 2013 | 1,2 | †∞ |
| Greece | Athens | 1997 | 1 | (29) |
| Hispaniola | | 2000 | 1 | (30) |
| India | Mumbai | 2009 | 1,3 | † ‡ |
| | Delhi | 2010 | 2 | (27) † |
| | Mumbai, Delhi | 2011 | 2 | (27) † |
| | Multiple | 2012 | 2 | (23) † |
| | Mumbai | 2012 | 1 | (23) † |
| | Bihar | 2013 | 1,2,3 | (23) † |
| | Mumbai | 2013 | 2 | (23) † |
| | Multiple | 2014 | 2 | † ‡ |
| | Vest Bengal | 2014 | 3 | † ‡ |
| Israel | Tel Aviv | 1998-2013 | 2 | (24) (27) § |

| | | | | |
|---|---|---|---|---|
| | Haifa | 2009-2012 | 1 | (23) |
| Nigeria | Multiple | 2011-2014 | 2 | (23),(30) † |
| Pakistan | Multiple | 2013-2014 | 2 | † |
| Slovakia | Bratislava | 2003 | 2 | (32) |
| | Skalica | 2003-2005 | 2 | (33) |
| South Africa | | 2001-2003 | 1, 2 | (34) |
| Switzerland | Zurich | 2008 | 1 | (24) |
| | Geneva | 2008 | 2 | (24) |

†Routine notification to WHO

∞National Institute for Health and Welfare (THL), Finland (unpublished observations).

‡Environmental Research Centre (ERC), India (unpublished observations).

§Central Virological Laboratory (CVL), Ministry of Health, Israel (unpublished observations).

**Table 2. Global detection of vaccine-derived polioviruses in sewage 19910-2014**

Systematic environmental surveillance for poliovirus circulation has been conducted in Egypt since 2000. During this time environmental surveillance has revealed:

- continued detection of wild polioviruses in sewage in the absence of detected AFP cases (6) (7);
- four independent importations of wild-type poliovirus between 2008 and 2012 (17, 35); and
- thirteen VDPVs detected between 2004 and 2013 from various locations in the north of the country(27).

In 2012, WPV was isolated from two sites in Cairo, although no WPV-confirmed AFP cases were detected, and no additional samples collected subsequently were found to be positive following enhanced sampling and immunization response activities (9).

In India, sampling of sewage helped to characterize the epidemiology of poliovirus circulation in settings where vaccination coverage was high yet paralytic polio cases continued to occur (18). Environmental surveillance in India continues to provide programmatically important information as WPV are no longer detected in either sewage samples or through AFP surveillance.

Environmental surveillance has become an important tool to supplement AFP surveillance in Pakistan and Nigeria, two countries where endemic WPV transmission has never been interrupted. In both countries, environmental surveillance has been established in large population centres and other strategic locations considered being at high risk of PV circulation. As in Egypt and India, PV isolates detected from environmental sewage sampling are sequenced to characterize their genetic relationships to other viruses from environmental samples and to other PVs isolated from AFP cases.

In Pakistan, environmental surveillance started in July 2009 with a dynamic expansion of the number and location of sampling sites. The 17 sites operating in 2011 were expanded to 23 in 2012, sampling major cities in 4 provinces. The additional sites have been established in locations where concerns over possible surveillance gaps have arisen. WPVs have been isolated frequently from sewage samples from all major cities in Pakistan since testing began, even in the absence of confirmed WPV cases detected through AFP surveillance. Samples from sites in some areas (e.g., Sindh, Punjab Provinces) have persistently yielded WPV isolates in the absence of WPV-positive AFP cases from the same area. Genetic sequencing of isolates from both AFP cases and sewage samples have been used to demonstrate epidemiological links and transmission routes allowing resources to be focused on the highest risk targets.

In Afghanistan, environmental surveillance was started in Kandahar Province in November 2013 and in Helmand Province in 2014.

Following an evaluation period and provision of training, environmental surveillance was started in Nigeria in July 2011. Monitoring was initially conducted in Kano, Northern Nigeria. Sites in additional States were added in 2012 (Sokoto and Lagos) and in 2013 (Kaduna, Federal Capital Territory, Borno, Kebbi and Katsina in order to characterize reservoirs of WPV and to better understand isolation of apparent 'orphan' viruses (isolates with $\geqslant$ 1.5% sequence diversity with their closely related isolate) in Northern Nigeria. Both WPV and VDPV have been isolated from samples collected at the majority of these sites, some in the absence of detected polio-positive AFP cases. In December 2012 sampling was extended to 4 additional sites in Lagos.

Numerous non-polio-endemic countries, including many in the WHO European Region, have also performed routine environmental surveillance of enteroviruses, including WPV and VDPVs, or have conducted research studies including environmental sampling for polioviruses (see ***Figure 1***)

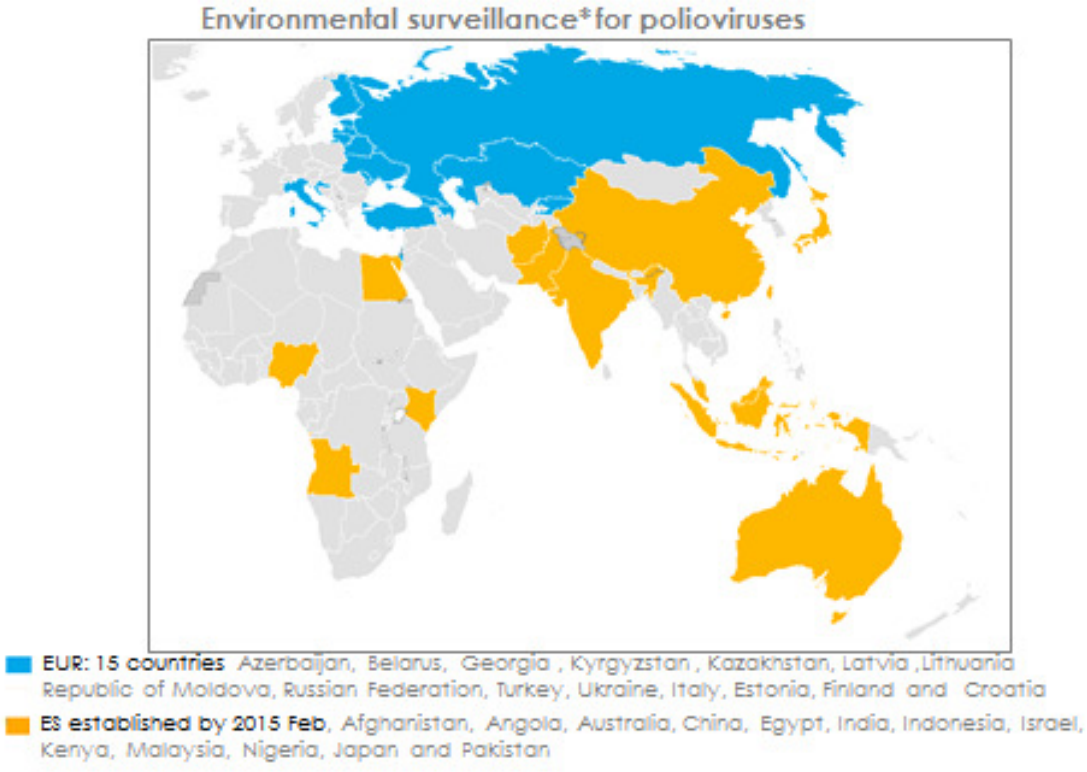


**Figure 1. Non-polio endemic countries performing routine environmental surveillance.**

## 3. Establishing the roles for environmental surveillance

### *3.1 Global priorities*

With global eradication approaching, environmental surveillance is being expanded to help identify some of the final reservoirs of WPV and to document eradication. For the GPEI the greatest priorities for developing and/or maintaining environmental surveillance are the remaining endemic countries (Nigeria, Pakistan and Afghanistan). Sensitive surveillance is also a high priority in polio-free districts adjacent to endemic districts (in the same or adjacent countries) and areas with recent or recurrent importation, re-establishment of transmission, or history of silent transmission despite the demonstration of adequate surveillance indicators.

### *3.2 Regional priorities*

Within the polio-free WHO regions, the use of environmental surveillance should be prioritized in areas at highest risk of WPV importation (or VDPV emergence) and spread, and those at risk of failing to detect WPV importation or VDPV emergence because of weak AFP surveillance. A key factor in evaluating the risk of PV outbreaks is the acknowledged difficulty in guaranteeing adequacy of AFP surveillance systems in detecting low-level PV circulation, with the potential for undetected 'silent' circulation. On the other hand, environmental surveillance is an equally important supplement for AFP surveillance in countries with very high vaccine coverage, since conditions may occur which allow for sustained high levels of silent PV circulation in the absence of any AFP (11, 4).The major risk for PV circulation relates to the level of susceptibility of the population to PV infection, but other important factors include the adequacy of water supplies and sanitation, level of mixing of infected persons with susceptible contacts, and other social/behavioural factors that favour the transmission of PV in a community. It is important to note that the risk status is dynamic and dependent upon changes in virus epidemiology, immunization coverage and surveillance status. The risk status of a country may shift significantly within a relatively short time period.

The identification of highest risk areas should be based on population characteristics, together with program performance indicators, and include consideration of:

- routine and supplementary vaccine coverage levels;
- the types of vaccine used in the vaccination programs (OPV, IPV, or OPV and IPV);
- AFP surveillance quality;
- areas/districts with a large floating population (migrants, nomads, refugees, informal settlements, undocumented guest workers);
- known or suspected population immunity gaps, such as adults and specific age cohorts that missed vaccination, and groups refusing vaccination on religious, philosophical or other grounds;
- districts with chronically poor surveillance performance indicators, and;
- the occurrence of large gatherings of people for commerce, religious or other occasions, especially where women and infants are included, such as specific events associated with holidays or festivals.

### *3.3 Priorities in a changing epidemiological landscape*

The role of environmental surveillance in support of the GPEI varies by setting and phase of the eradication program. A country can be categorized according to the current epidemiological status of PV circulation. These categories include:

- Countries that have never interrupted virus circulation and retain widespread or focal endemic transmission;
- Countries that have interrupted endemic virus circulation but are affected by re-establishment of transmission following importation;
- Currently polio-free countries that are considered to be at risk of a polio outbreak following WPV importation or emergence of neurovirulent vaccine-derived poliovirus.

### 3.3.1 Role in endemic/re-established transmission areas

In endemic settings, environmental surveillance can complement AFP surveillance and help monitor the genetic diversity of isolated PVs. Genetic sequencing of isolates may allow the linking of a viral isolate to other circulating PVs and help differentiate between outbreaks associated with indigenous PV stains and those that may occur following introduction of virus from other endemic areas. Sequence analysis can also identify the location from which a poliovirus was imported, i.e. where the poliovirus is presumably still circulating and where intervention is required to break the chains of transmission. In a PV-endemic setting the considerable resources required for the field and laboratory components of environmental surveillance must be balanced against the resource needs for maintaining high-quality case-based AFP surveillance and attaining full vaccination coverage. The GPEI strongly advises that existing resources used for AFP surveillance should not be reallocated to environmental surveillance.

### 3.3.2 Role in areas with recent WPV circulation

Polio environmental surveillance can help to verify that circulation of WPV has stopped in an area; but environmental surveillance results must be interpreted in conjunction with highly sensitive AFP surveillance and cannot replace AFP surveillance. Identification of PV from environmental sampling sites in the absence of cases may indicate less than adequate AFP surveillance and continued PV circulation in the absence of cases. Genetic sequencing of PVs from environmental samples can help determine if the isolates represent longer-standing 'silent' local circulation or result from new importations.

### 3.3.3 Role in areas at high risk of poliovirus outbreak following importation

Rapid detection of imported WPV is a significant reason for considering establishing polio environmental surveillance in areas at high risk of importation and subsequent outbreak. Re-emergence of WPV poliomyelitis in many previously polio-free countries demonstrates that herd immunity in some polio-free developing countries may be extremely fragile (2). Even temporary deficits in vaccination coverage can sometimes be sufficient to facilitate circulation of imported WPV (36). Evaluating the risk of an outbreak following importation is not an exact science, and should be conducted through consultation between the country program and WHO country and regional offices using the best available evidence. It should be noted that the frequency of environmental sampling (weekly/monthly/bi-monthly) at a given site affects the potential rapidity with which environmental surveillance and detect putative virus circulation. Furthermore, the longer that routine surveillance is continued at a given site, the easier it is establish base lines for recognizing the sudden appearance of unusual events.

### 3.3.4 Role in low risk areas

Recognising that risk status is dynamic and may change dramatically in a short period of time, 'low risk' areas may be defined as those unlikely to experience a polio outbreak because of high levels of population immunity, low likelihood of widespread transmission, and sensitive case-based disease surveillance systems. In these situations, even though environmental surveillance may be useful for detecting VDPVs and for monitoring potential circulation of Sabin-related viruses following OPV use, priority should be given to sustain good immunization and AFP surveillance systems. In settings

where AFP surveillance can be demonstrated to meet certification quality standards and vaccine-coverage is high, environmental surveillance is generally considered ***unlikely*** to add much to the sensitivity of the system. Exceptions to this may be in populations with documented high level immunity to poliomyelitis due to exclusive inactivated polio vaccine (IPV) use that are frequently exposed to importations. Poliovirus infection in these populations is far less likely to result in clinical paralytic polio cases (37), and so not be detected by AFP surveillance systems. This has recently been demonstrated in Israel (4), where environmental surveillance has demonstrated the widespread transmission of imported PV in the absence of detected clinical cases.

In settings where risk is considered low, national programs interested in carrying out environmental surveillance may face challenges in justifying the additional resources needed, particularly in competition with other health priorities. It becomes essential, therefore, to clearly define the expected role and outcomes of environmental surveillance in the context of country and Regional priorities (e.g., Regional certification).

#### 3.3.5 Role in other specific settings or populations

*Specific populations*

Other potential settings or populations for environmental surveillance of PVs include schools or social/health child-care settings, or special populations, such as refugee camps. Locations that have high concentrations of young children and centralized sewage systems may present an opportunity to monitor PV. Refugee camps may provide an opportunity to monitor excretion of PV if sewage collection processes are adequate. Reuse of wastewater for agriculture and other purposes is increasing globally and environmental surveillance has a potential role for ensuring the safety of this reclaimed water. These special settings/populations may not necessarily represent the larger populations around them in terms of immunity through vaccination. This should be taken into consideration for the interpretation of any results and in planning program actions to limit susceptibility to PV infections as well as other vaccine-preventable diseases.

*Areas where the risk of failure of PV containment is high*

Laboratories or vaccine development settings that store PVs, use PVs for research purposes, or manufacture IPV must follow recommended containment strategies to avoid the risk of poliovirus transmission[2]. Areas where there is a risk of failure to properly contain polioviruses are at risk of re-introduction of PVs into the community. Environmental surveillance in these settings would help to monitor for PV 'escapes' and provide information for prevention of circulation or outbreaks.

### *3.4 Role within the polio 'endgame' strategy*

The work of the GPEI will not end once polio has been eradicated. Activities will be needed to minimize the risks of PV re-introduction and the emergence of cVDPVs. To prepare for the management of these risks, the GPEI has developed a multi-pronged programme of work consisting of research, new product development, strategy formulation and policy development. *The Polio Eradication and Endgame Strategic Plan 2013–2018* is a comprehensive, long-term strategy that addresses what is needed to deliver a polio-free world by 2018. The Plan has four objectives:

1. Detect and interrupt all poliovirus transmission - stop all WPV transmission by the end of 2014 and new cVDPV outbreaks within 120 days of confirmation of the first case;

[2] WHO global action plan to minimize poliovirus facility-associated risk after eradication of wild polioviruses and cessation of routine OPV use (DRAFT 2009). Available online: http://www.polioeradication.org/Portals/0/Document/Resources/PostEradication/GAP3_2009.pdf

2. Strengthen immunization systems and withdraw of OPV, beginning with the withdrawal of the type 2 component of trivalent oral polio vaccine (tOPV) leading to eventual withdrawal of all OPV;
3. Contain poliovirus and certify interruption of transmission - certify all regions of the world polio-free and ensure that all poliovirus stocks are safely contained by 2018;
4. Plan polio's legacy - ensure that a polio-free world is permanent and that the investment in polio eradication provides public health dividends for years to come.

Environmental surveillance clearly has a potential role to play in meeting these objectives, particularly the detection of ongoing circulation of WPV and cVDPVs, monitoring the disappearance of Sabin-related viruses, monitoring around PV containment facilities and providing evidence for global certification.

## 4. Principles for selecting sampling sites

### *4.1 Selection of sampling sites appropriate for the target population*

A major factor limiting the wider application of effective environmental surveillance is the lack of sewer networks in some of the highest priority areas. This poses a significant challenge for authorities attempting to identify representative sampling sites. In most circumstances it is necessary to consult with the local sanitary engineering authorities to assess the possibilities for collection of representative samples derived from the target population.

For systematic environmental PV surveillance it is optimal but not obligatory that most households are equipped with water closets connected to a converging sewer network allowing collection of downstream samples that represent a large number of people living in the catchment area. Recommended sampling sites are inlets to sewage treatment plants or other major collector sewers. Industrial wastes may contain compounds that may be toxic to cell cultures and/or interfere with poliovirus replication. This must be taken into account when selecting the sampling sites.

Other systems for wastewater flow, such as open canals or water channels, have in some cases enabled successful demonstration of wild poliovirus circulation in the relevant population (18). In the absence of a sewer network, representative sampling may be difficult to achieve and environmental surveillance should only be started if the major flow routes of wastewater containing human faecal material are sufficiently well known. Sampling in such situations may result in lower surveillance sensitivity due to a variety of undefined or uncontrolled factors, such as environmental inactivation of viruses. Targeted, carefully designed stool surveys may be considered as an alternative approach to environmental surveillance in the absence of a sewer network.

### *4.2 Size of population to be sampled*

The number of people living in the catchment area affects the sensitivity of PV detection in a population in two ways: by increasing the area it is possible to monitor more people with fewer samples and increase the probability of detecting low level transmission. On the other hand, this increase is likely to diminish the sample sensitivity, i.e. capacity to detect small numbers of PV excretors in the population as the increasing number of non-excretors may dilute the virus to below the limits of detection. In practice, the size of the source population in established systems where WPV and VDPV have been detected has varied from tens of thousands to a few millions (2).

Sampling sites chosen for regular monitoring should represent selected high-risk populations with a preferable size of 100,000 to 300,000 persons. If it is more feasible to divide the target population to smaller subpopulations and the sampling sites are close to each other, generation of composite samples by mixing portions derived from different sites can be considered to reduce the laboratory workload. If the source population is larger, the consequently reduced sample sensitivity can be compensated for by collecting more frequent samples, acknowledging, however, that this would necessarily increase the laboratory workload.

### *4.3 Sampling site sensitivity*

A mathematical model has been established to help visualize the different factors influencing the proportion of excreted PV that can be recovered at a downstream sampling site, and so estimate the sample and population sensitivities (38). This model demonstrates how the different factors affecting the sensitivity of the surveillance approach are related. For example, how less than optimal virus detection sensitivity could be compensated for by collecting larger volumes for analysis, and for detection of emerging outbreaks of virus circulation, frequently repeated sampling is critical.

Increasing the size of the source population may decrease the sample sensitivity, by effectively decreasing the concentration of virus in the wastewater samples collected. In some situations, therefore, a larger number of poliovirus-infected individuals may be needed for a given sample to reveal poliovirus. In large cities, it may be necessary to segment the population and to sample preferred subgroups within the segment. Appendix 2 gives further details and considerations to estimate the sample sensitivity in different situations.

## 5. Programmatic considerations for the inclusion of environmental surveillance in a national plan for poliovirus surveillance

### *5.1 Revising the national plan*

If environmental surveillance in support of the GPEI is being considered it should be integrated into the national plan for poliovirus surveillance. Development of this element in the plan should exploit different expertise including the national Expanded Programme on Immunization (EPI), national polio laboratory (NPL), local sanitary engineering authorities, and other relevant provincial and local authorities. The WHO regional office should be consulted at an early phase of the planning.

With regard to environmental surveillance, the plan should include the following elements:

- length and time schedule of sampling;
- details of the actual sampling sites (location, GPS coordinates if available, population sizes and demographics likely to be represented);
- responsibilities for sampling, instructions for sampling and sample logistics;
- provision of adequate laboratory space, personnel, equipment and reagents;
- standardized protocols for sample collection, transportation, processing and virus identification;
- data management and reporting systems (contents of reports and reporting channels);
- training and quality assurance;
- discussion of programmatic responses to both positive and negative laboratory findings.

Within each WHO region, countries identified with prioritized locations should also adhere to the guidance described in this document for assessing the optimal sampling sites and assessing the feasibility for laboratory processing of samples. Options for the establishment of central or regional processing laboratories should be considered, taking into account the transportation and reverse cold chain needs. The need to meet national border control requirements and IATA regulations for shipment must also be considered for international transport.

### *5.2 Budgeting for environmental surveillance*

The processing and analysis of environmental specimens create a significant workload and cost increase for the laboratory. The establishment and running costs of an environmental sampling laboratory are high. The GPEI has calculated that, for a laboratory already involved in PV isolation from clinical specimens, investment of an additional US$ 50,000 would be required for equipment and consumables to process environmental samples. A further US$ 33,000 would be required to cover the costs of analysing 100 samples (2). Additional salaries and training for laboratory staff also need to be considered in the cost estimates for planning. According to an almost 10-year experience in Egypt, processing and analysis of 100 sewage samples in the laboratory requires trained staff at approximately the same level as for processing and analysis of stools from 200 AFP cases with two specimens from each (2). Program managers must ensure that laboratory performance does not suffer because of the increased resource requirements presented by environmental surveillance activities.

### *5.3 Length and time schedule of sampling in different situations*

As noted above, environmental surveillance can be instituted for different purposes and in different settings.

- If aimed at providing supplementary evidence for elimination of WPV circulation in a population, a long term, regular sampling programme of a representative population is

preferable. Sampling frequency should, preferably, be ***weekly*** or ***biweekly***, but at least once per month. Sampling should be continued for at least one year, and preferably three years after the last wild poliovirus isolation.

- If environmental surveillance is prompted by known or suspected reintroduction of WPV or appearance of cases caused by circulating cVDPV, the initial plan may cover a shorter period (not less than 12 months) and apply more frequent sampling, targeted to more selected populations. This must always be accompanied by intensified AFP surveillance.

When monitoring large populations served by a complex sewer network, weekly grab samples during the hours of peak usage are likely to be sufficient as transport through the sewer pipes of any input virus is likely to be partially delayed, and virus concentration at the sampling site is not affected by toilet-use frequency of the source population (39). The closer the sampling site is to the source, the higher the probability of detection. However, if the sampling site is very close to a suspect population, samples may not be sufficiently dispersed and a composite sampling system, i.e. 24-hour pooled samples composed of hourly collected aliquots, may be necessary in order not to miss the peak virus concentrations in the sewage (2).

In all situations sampling dates should be coordinated with officials in charge of logistics and with the NPL to avoid unnecessary storage and transport delays of the collected samples before processing can be arranged. An example timetable for the collection of environmental samples from different sites is provided in ***Figure 2***.

| S.No | Town | Sites | UC/areas drained by | ID CODE | First Week | | | | | | | Second Week | | | | | | | Third Week | | | | | | | Fourth Week | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | W | T | F | S | S | M | T | W | T | F | S | S | M | T | W | T | F | S | S | M | T | W | T | F | S | S | M | T | W | T |
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 1 | Town A | Site 1 | a* | GI-1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | | Site 2 | b* | GI-2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | | ** | c* | GI-3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | Town B | Site 1 | d* | GP-1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | | Site 2 | e* | GP-2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | Town C | Site 1 | f* | BD-1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | | Site 2 | g* Temporarily Closed | BD-2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | | Site 3 | h* | BD-3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Total** | **3** | **5** | | | **2** | | | | | | | **1** | | | | | | | **1** | | | | | | | **2** | | | | | | **6** | |

| | |
|---|---|
| | ID Code |
| Key | |

ID CODE= PAK/SD/KCH/xxx/09/xxx

Notes:

1. Each collection site has a unique ID Code and is consistent with the country epidemiological ID number and includes: country, province, city, etc. Environmental IDs should have a different format from EPIDs. For example, containing an extra 3-letter code and /or a tag such as “env”

2. Each site has the geographic drainage areas defined and listed (a*-h*)

3. Each sample collected is noted on the timeline by week and date of collection.

**Figure 2. Example of a timetable for collection of environmental samples from multiple sites.**

### *5.4 Sampling principles and sample logistics*

The plan should clearly indicate who is responsible for collecting the samples at each sampling site. Sampling can be organized by the local authorities or centrally, through the national health authorities or NPL, whichever is considered the most suitable alternative for the particular situation. Exploiting the use of an existing sewage sample collection system should be considered whenever possible. Training and written instructions should be provided to persons collecting the samples. An example Standard Operating Procedure (SOP) is given in Appendix 5.

There are two principal modes of collecting environmental samples for virological analysis, referred to as grab and trap sampling.

#### 5.4.1 Grab sampling

In the grab method an amount of raw sewage is collected at a selected sampling site, either at one point in time, or, depending on the complexity of the sewage network and size of population being sampled, at different predetermined times to form a time-adjusted composite sample. Many sewage treatment plants use automated equipment for collecting samples at regular intervals during a 24-hour period or over the peak hours of household sewage flow. In addition to using automatic composite samplers located at the entry into sewage processing plants, it is also possible to use portable computerized automatic samplers that can be located at upstream branches of sewage systems at timed intervals over a 24 hour period. Manual collection of composite samples is also possible if automated collectors are not available but sustained adherence to the relatively tedious collection practice may be difficult to guarantee. If a complex network serving a large target population is being sampled, single time-point samples, collected automatically or manually, may be preferable.

Grab sample volumes of one litre are recommended. The larger the volume of sewage analysed, the higher the theoretical sensitivity to detect poliovirus circulation in the source population. In practice volumes larger than one litre are difficult to handle in the laboratory and may be replaced by several parallel regular samples. Larger volumes or parallel samples mean, however, increasing laboratory time and workload per site, and may limit the number of sites that can be monitored. A practical bottleneck in most laboratories is the virus detection phase. Using cell culture inoculation, only a small part of the material concentrated from 1 litre wastewater can be used for virus testing.

In special circumstances it may be important to quantify the amount of poliovirus in the environmental samples (4, 40, 41). For this it is especially important to use reproducible standard operating procedures for collection and processing of the sewage.

#### 5.4.2 Trap sampling

Trap samples are collected by hanging a bag of non-specifically absorbing material in the sewage stream. After one or more days, the bag is taken out of the sewage and shipped to the laboratory, where the absorbed material is eluted and analysed for (polio)viruses. Although trap sampling has been used successfully for many years in some countries, grab sampling is preferred to trap sampling as it is more feasible for quantitative estimation of detection sensitivity of the system, and long-term experience suggests that programmes exploiting concentrated grab samples detect polioviruses and non-polio enteroviruses more often than those using trap sampling.

#### 5.4.3 Time of sample collection

Time of sample collection can be very important, particularly when samples are collected from open drains/canals. Time of collection should be selected based on expected maximum affluent flow from communities, which is often between 6-8 am. Time of sampling also becomes important when the temperature is high, as viruses are expected to be inactivated more rapidly.

### 5.4.4 Sample shipment

Whatever the sampling principle, collected samples should be immediately refrigerated and kept cool during transport to arrive at the processing laboratory within 48 hours of collection. The laboratory should be notified in advance and the laboratory should acknowledge the receipt of the sample. An example laboratory request form is provided in ***Figure 3***.

**LABORATORY REQUEST FORM**
**For Collected Wastewater Sample for Environmental Surveillance**
(To accompany sample being sent to laboratory for analysis)

| |
|---|
| Country: ID Code: / / / |
| Name of the site: |
| Atmospheric temperature at time of sample collection: ºC |
| Type of sewage plant or sewage system: "Open Drain" "Sewage Pump" |
| Date of sample collection |
| Time of sample collection |
| Date of receiving sample in MoH/WHO office: |
| Date of sending sample to laboratory: |
| Name of the person who collected sample: Contact # |
| Name & Designation of person filling form: Contact # |
| Name & Designation of focal person /supervisor: _____Contact #_ <br> Signature:_____ Date:_____ |
| |
| **(FOR USE BY LABORATORY )** <br> Date of sample received at laboratory: Day_____Month_____Year_____ <br> Sample Lab. No._____ <br> Condition of sample at receipt: ---- Good ---- Poor <br> Name of person receiving sample at laboratory:_____ |

**Figure 3. Example of a specimen collection form**

### *5.5 Sample processing in the laboratory*

The processing of both grab and trap samples in the laboratory contains steps that may generate aerosols, and all precautions must be taken to avoid exposure of laboratory personnel to infectious agents, according to guidelines provided in the WHO Laboratory biosafety manual[3]. Laboratory quality assurance practices and procedures, as described in the WHO Polio laboratory manual[4] should be followed by all laboratories testing environmental samples. At a minimum, all laboratory facilities processing environmental samples should meet basic WHO BSL-2 laboratory standards (***Figure 4***). Note that according to these recommendations all steps of the 2-phase extraction procedure should be performed within a Biological safety cabinet.

**Minimum standards for a WHO Biosafety Level 2 laboratory**

- Ample space is provided for the safe conduct of laboratory work and for cleaning and maintenance
- Walls, ceilings and floors are easily cleaned
- Illumination is adequate for all activities
- Storage space is adequate to hold supplies for immediate use
- Hand washbasins, with running water, if possible, are provided in each laboratory room, preferably near the door
- An autoclave (or suitable pressure cooker is available in the same building as the laboratory
- Facilities for storing outer garments and personal items for eating and drinking are provided outside the working areas
- A good quality and dependable water supply is available. There are no cross-connections between sources of laboratory and drinking water supplies
- A standby generator is desirable for the support of essential equipment such as incubators, biological safety cabinets, freezers and the like
- Pipetting aids are available to replace mouth-pipetting
- Biological safety cabinets are available for:
  - Procedures with high potential for producing aerosols, including centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, and opening of infectious materials whose internal pressure may be different from ambient pressure
  - Handling high concentrations or large volumes of infectious materials
- Centrifuges with sealed safety caps are available or centrifuging high concentrations or large volumes of infectious materials in the open laboratory. These caps must be loaded and unloaded in a biological safety cabinet
- Screw-capped tubes and bottles are available to hold positive specimens and cultures
- Autoclaves are available to sterilize contaminated material

**Figure 4. The Basic Biosafety Level 2 (BSL-2) facility** (from the WHO Polio laboratory manual – 4th edition)

Processing and analysis of environmental samples must not interfere with the processing and analysis of samples collected from AFP patients and their contacts. Similarly, environmental

[3] *WHO Laboratory biosafety manual – 3rd edition* (WHO/CDS/CSR/LYO/2004.11). Available online: http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

[4] *WHO Polio laboratory manual – 4th edition (WHO/IVB/04.10).* Available online: http://www.who.int/immunization/documents/WHO_IVB_04.10/en/index.html

specimens or their processing must not be exposed to potential poliovirus contamination from faecal specimens or from laboratory stock preparations, which may have PV titres several magnitudes higher than those found in wastewater. It is recommended that separate laboratory space and personnel should be assigned for work with environmental samples and AFP samples.

### 5.5.1 Processing fluid grab samples in the laboratory

Half of the collected raw sewage specimens i.e. 500 ml should be concentrated before inoculation into cell cultures to improve detection sensitivity. The other half should be kept at -20°C as a backup until the final results of the concentrate from the first half are available. The first step in any concentration procedure is clarification of the sample, i.e. pelleting of larger suspended solids by centrifugation (see ***Appendix 1***). PV may be partly bound to these solids so the pellet should be processed separately, and the extract included in the analysis.

One frequently used method for sewage sample concentration is the two-phase separation method (see ***Appendix 1***). The resulting nominal sample concentration is approximately 50-fold. This method is simple enough to be adopted in any NPL provided that necessary training has been given, the equipment is available and sustained provision of the reagents can be guaranteed.

Several alternative methods have been used to successfully concentrate sewage samples but these methods have yet to be fully validated. Two methods that have been well-documented include precipitation with polyethylene glycol (PEG) and ultrafiltration. Using either of these methods, a more than 100-fold nominal concentration can be obtained, but this should be viewed with caution for sewage specimens because (i) toxic compounds may also be concentrated, (ii) recovery of the virus from the concentrate does not increase proportionally to the nominal concentration, and (iii) if higher nominal concentration results in inoculation of a smaller number of cell culture vials, separation of virus mixtures may become more complicated.

## *5.6 Detection of poliovirus in environmental samples*

In principle, environmental sample concentrates and trap eluates are examined for the presence of PV in the same way as faecal specimens (see the *WHO Polio laboratory manual, 4$^{th}$ edition*). Because of the specific nature of the specimens, some modifications are, however, recommended. For possible confirmatory tests at a later stage, one quarter of the processed sample (at least 3 ml) should be stored frozen at -20°C. 20 % of the remainder (corresponding 100 ml of raw sewage) should be inoculated into five L20B and one RD cell cultures (Appendix 1 for details).

Maintenance and follow-up of the inoculated cultures are similar to those inoculated with clinical specimens (see *WHO Polio Laboratory Manual, 4$^{th}$ edition),* with observation time of 5 days maximum, cross-passaging RD positive isolates in L20B cells, followed by enrichment of all L20B positive isolates in RD cells. After enrichment all isolates of $L^+$ $R^+$ -arm are subjected to intratypic differentiation (ITD) by real-time reverse transcription polymerase chain reaction (rRT-PCR). L20B cell isolates enriched in RD cells are prioritized for ITD as environmental specimens frequently contain mixtures of non-polio enterovirus types that are difficult to resolve.

Using the bulk culture approach there is a risk of missing low-concentration and low-fitness components in a virus mixture. Using multiple parallel cultures, e.g. 5 x L20B flasks, may partially overcome this problem but considerably increases laboratory workload and costs. Using a plaque assay to isolate individual virus strains from environmental specimens has been shown to be a useful means to avoid this risk. This technique, however, is tedious, time-consuming and labour-intensive.

## *5.7 Characterization of poliovirus isolates*

To meet GPEI requirements all PV isolates from environmental samples should be differentiated as WPV, SL, or VDPV in a WHO-accredited ITD laboratory (see *WHO Polio Laboratory Manual, 4$^{th}$ edition*). The agreed turnaround time for laboratory procedures are as follows:

- Concentration and isolation procedures - 21 days
- ITD - 7 days
- Shipment of isolates - 7 days
- Sequencing - 14 days

Laboratories, however, should in general give highest priority to characterizing polioviruses obtained from AFP cases and their contacts. Exceptions may be justified in specific epidemiological situations, for example, when suspecting an emerging outbreak due to importation of WPV or VDPV . Environmental surveillance is likely to generate a substantial workload for ITD tests because of the likely preponderance of Sabin polioviruses in countries where OPV is used. Therefore logistic arrangements for ITD tests must be incorporated in the planning stages. It is noteworthy that parallel poliovirus isolates derived from a given environmental specimen may not be identical even if belonging to the same serotype, and may contain a mixture of SL and non-Sabin-like (NSL) strains. A backup portion of the sample should be kept at the first-level testing laboratory to be made available to a second-level testing laboratory for repeat testing as necessary.

PV isolates showing discordant rRT-PCR ITD results may represent VDPVs and should be sequenced for further characterization, as it is done for clinical isolates. It is important to always keep in mind that poliovirus mixtures are common in environmental specimens, which may cause confusion in interpreting ITD results and might require additional sequencing reactions to identify components of the mixture.

### *5.8 Reporting laboratory results*

Reporting of laboratory results from environmental surveillance to national health authorities and WHO should follow the guidelines of reporting for clinical surveillance with respect to the need for regular reporting of activities and findings as well as immediate reporting of WPV or VDPV isolation. As environmental surveillance of polioviruses often involves personnel from other departments, such as the ministry of environment, sharing periodic reports among government departments is highly recommended.

### *5.9 Interpretation of results and programmatic consequences*

The route of poliovirus from an infected individual through the environment to the cell cultures at NPL is very complex, and thus the results obtained in environmental surveillance should be interpreted with caution. A useful criterion of satisfactory overall performance of the surveillance system is detection of non-polio enteroviruses in the samples. It is not possible to give one single figure for the expected percentage of non-polio enterovirus (NPEV) positive environmental samples because geographical location, climate, population density and many other factors influence it (see Appendix 1). A common sense view is that the percentage should usually be at least as high as that in faecal specimens from AFP patients in the same population. In populations immunized with OPV, environmental surveillance should also reveal SL strains, especially during and immediately after SIAs and other supplementary immunization activities.

Abundant OPV-related strains in the sewage may theoretically mask the presence of small amounts of WPV if the standard techniques are being used without specific selective conditions for wild virus. However, there is substantial evidence from successful isolation of WPV during and immediately after SIAs, indicating there is no need to interrupt environmental surveillance because of an OPV campaign.

Isolation of WPV from an environmental sample should raise the same questions and result in similar actions to those resulting from detection of a WPV-associated AFP cases, i.e. a determination should be made of whether the result represents very recent importation of the virus or widespread WPV circulation within the community. The immediate response should be intensified AFP surveillance in the community, more frequent and possibly redesigned environmental sampling, and preparation

for supplementary immunization activities. Environmental findings should be assessed in the context of all other epidemiological information. Both the WHO regional and headquarters offices should be consulted on proposed programmatic actions.

Isolation of WPV from an environmental sample usually indicates that a number of individuals are excreting the virus. It is theoretically possible, however, to detect excreted virus from a single person importing the virus into a polio-free population. For this reason it is important to rapidly characterize the isolated virus and to repeat the sampling in order to interpret the epidemiologic significance of virus detection and develop an appropriate programmatic response.

Swiss public health authorities reported the isolation of a WPV from sewage water collected in Geneva in August 2007. Due to high vaccination coverage and good sanitation, the isolation was considered to represent no significant risk of outbreak for Switzerland. The virus was genetically closely related to WPV detected in Chad at that time. The Swiss authorities heightened surveillance and assessment of polio immunisation coverage of communities where the virus was detected and in surrounding areas. No cases of paralytic polio and no additional WPV-positive samples were detected (22). Similarly, rapid partial sequence analysis of all non-Sabin-like isolates revealing iVDPV-like strains in sewage of Tallinn, Estonia (25) and Tampere, Finland (28), helped in making decisions not to mount extensive supplementary immunization activities.

Negative results are more difficult to interpret and should be assessed in relation to the sampling design and efficiency of laboratory procedures. Samples that test negative for PV may indicate the absence of circulating virus, or test negative for a range of other reasons:

- a poorly- or improperly-selected collection site;
- inadequate or inappropriate sample collection methods;
- inadequate sample transport or storage;
- inadequate or inappropriate sample concentration method;
- low sensitivity of the laboratory methods used for virus detection and identification;
- presence in the sewage of substances toxic for cell cultures;
- co-extraction of inhibitors of RT-PCR.

The theoretical maximum sample sensitivity can be calculated by using some assumptions (See ***Appendix 2***). Repeated sampling will increase the probability of detecting low-level transmission of WPV or cVDPV in a population. If a population is monitored using the recommended methods with acceptable quality indicators, the finding of consistently negative wild poliovirus results for 12 months suggest that wild poliovirus is not circulating in the population. If this situation continues for three successive years, wild poliovirus circulation is highly unlikely in the source population. These conclusions should be drawn with caution if there is a high risk of importation of wild poliovirus.

Examples of the reporting of results as maps and report forms are shown in ***Figure 5, Figure 6*** and ***Figure 7***.

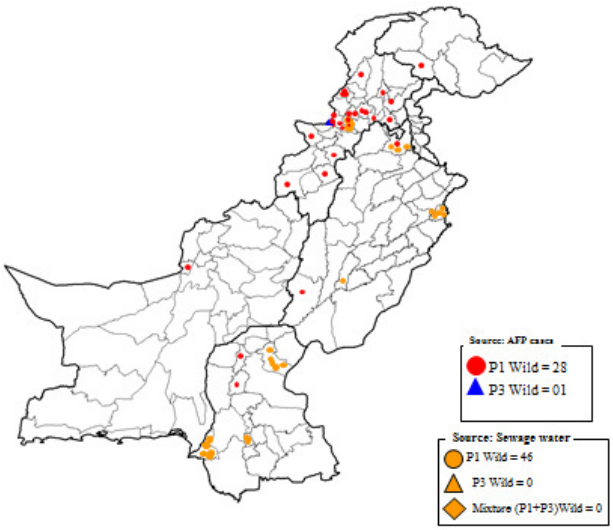


**Figure 5. Map of the locations in Pakistan where WPV detected from stool samples of AFP cases and from Sewage water.**

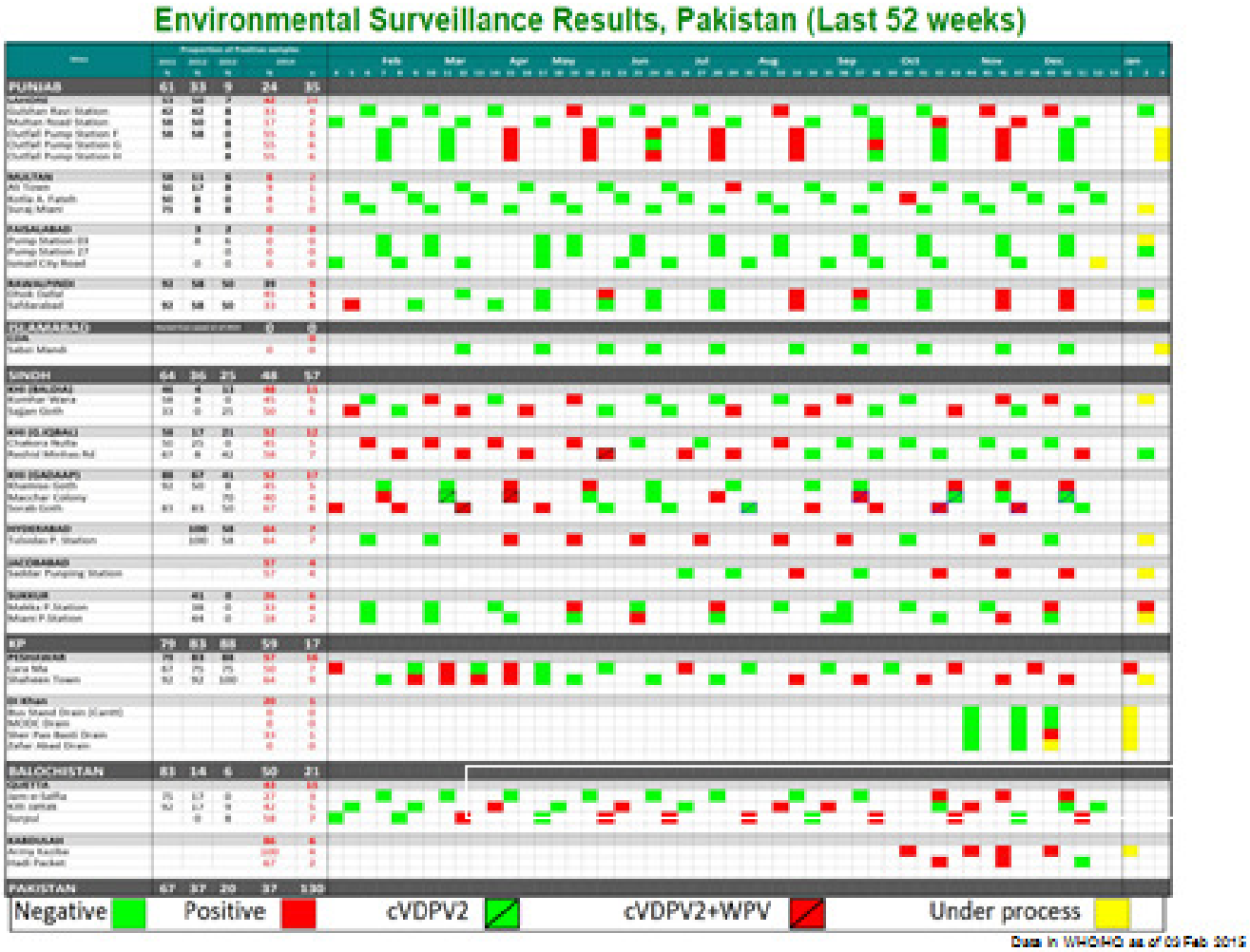


**Figure 6. Summary of Laboratory data for Environmental Surveillance- Pakistan. Report for Epidemiological week= 40 (30 Sep – 06 Oct, 2012).**

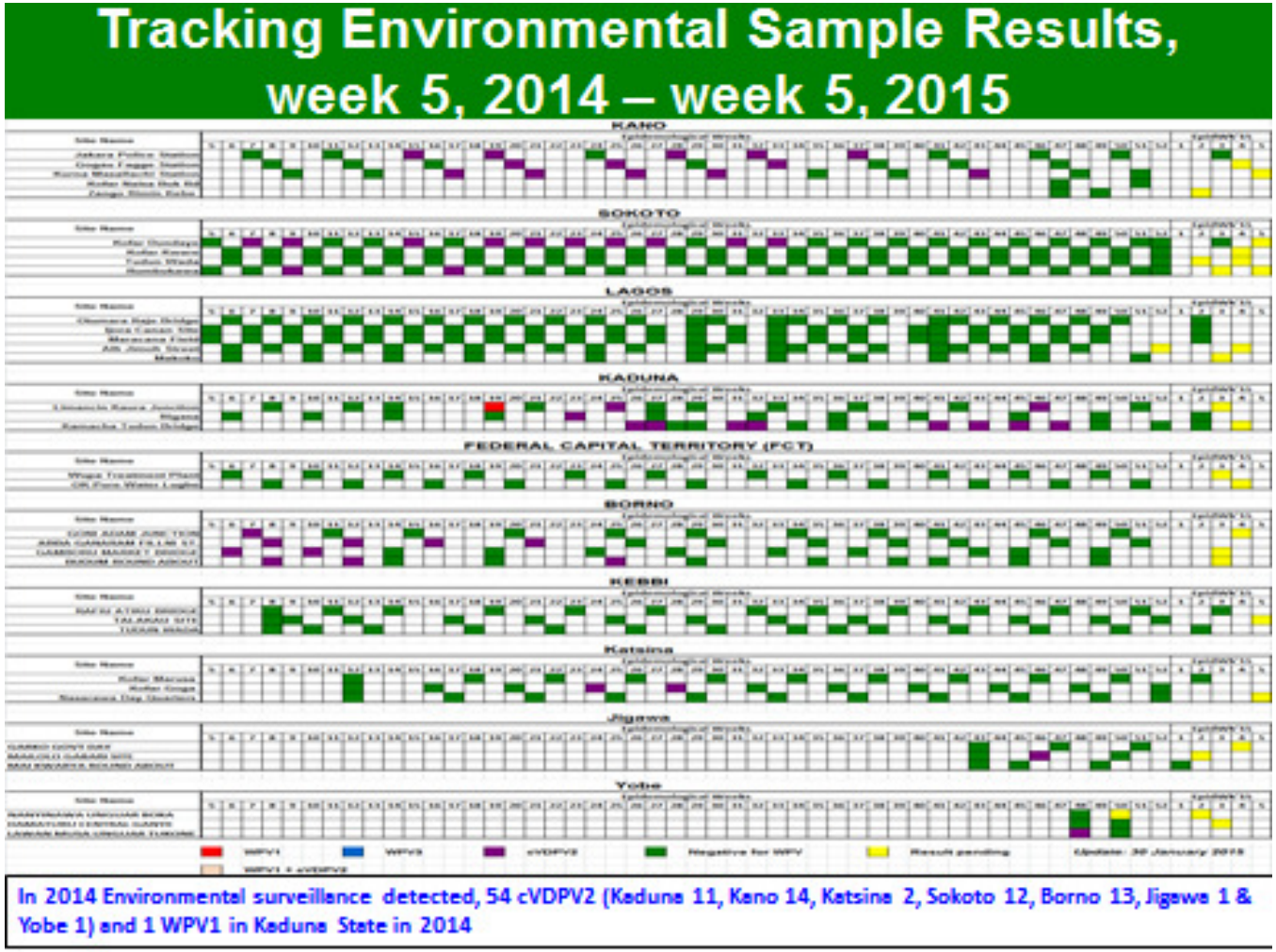


**Figure 7. Results of environmental surveillance in Nigeria from 2011-2012 .**

## References:


1. Dowdle, W. et al. , *Containment of polioviruses after eradication and OPV cessation: characterizing risks to improve management*. Risk Anal, 2006. **26**(6): p. 1449-1469.

2. Hovi, T. et al. , *Role of environmental poliovirus surveillance in global polio eradication and beyond*. Epidemiol Infect, 2012. **140**(1): p. 1-13 LID - 10.1017/S09.

3. Dowdle, W. R. and M. E. Birmingham, *The biologic principles of poliovirus eradication*. J Infect Dis, 1997. **175 Suppl 1**: p. S286-92.

4. Manor, Y. et al. , *Intensified environmental surveillance supporting the response to wild poliovirus type 1 silent circulation in Israel, 2013. LID - 20708 [pii]*. Euro Surveill, 2014. **19**(7).

5. CDC, *Tracking progress toward global polio eradication--worldwide, 2009-2010*. MMWR Morb Mortal Wkly Rep, 2011. **60**(14): p. 441-445.

6. El Bassioni, L. et al. , *Prolonged detection of indigenous wild polioviruses in sewage from communities in Egypt*. Am J Epidemiol, 2003. **158**(8): p. 807-815.

7. Hovi, T. et al. , *Environmental surveillance of wild poliovirus circulation in Egypt--balancing between detection sensitivity and workload*. J Virol Methods, 2005. **126**(1-2): p. 127-134.

8. Shukla, D. et al. , *Environmental surveillance of enterovirus in Northern India using an integrated shell vial culture with a semi-nested RT PCR and partial sequencing of the VP1 gene*. J Med Virol, 2013. **85**(3): p. 505-11 LID - 10.1002/jmv.

9. Chowdhary, R. and T. N. Dhole, *Interrupting wild poliovirus transmission using oral poliovirus vaccine: environmental surveillance in high-risks area of India*. J Med Virol, 2008. **80**(8): p. 1477-88 LID - 10.1002/jmv.

10. WHO, *Outbreak news. Poliovirus isolation, Egypt*. Wkly Epidemiol Rec, 2013. **88**(7): p. 74-75.

11. Anis, E. et al. , *Insidious reintroduction of wild poliovirus into Israel, 2013. LID - 20586 [pii]*. Euro Surveill, 2013. **18**(38).

12. Shulman, L. M. et al. , *Bioterrorism and Surveillance for Infectious Diseases - Lessons from Poliovirus and Enteric Virus Surveillance*. J Bioterr Biodef, 2012. **S4:**004.

13. Manor, Y. et al. , *A double-selective tissue culture system for isolation of wild-type poliovirus from sewage applied in a long-term environmental surveillance*. Appl Environ Microbiol, 1999. **65**(4): p. 1794-1797.

14. Manor, Y. et al. , *Advanced environmental surveillance and molecular analyses indicate separate importations rather than endemic circulation of wild type 1 poliovirus in Gaza district in 2002*. Appl Environ Microbiol, 2007. **73**(18): p. 5954-5958.

15. Tambini, G. et al. , *Direct detection of wild poliovirus circulation by stool surveys of healthy children and analysis of community wastewater*. J Infect Dis, 1993. **168**(6): p. 1510-1514.

16. WHO, *Global detection of wild and vaccine derived polioviruses, January 2008-June 2009*. Wkly Epidemiol Rec, 2009. **84**(36): p. 366-370.

17. Blomqvist, S. et al. , *Detection of imported wild polioviruses and of vaccine-derived polioviruses by environmental surveillance in Egypt*. Appl Environ Microbiol, 2012. **78**(15): p. 5406-9 LID - 10.1128/AEM..

18. Deshpande, J. M., S. J. Shetty and Z. A. Siddiqui, *Environmental surveillance system to track wild poliovirus transmission*. Appl Environ Microbiol, 2003. **69**(5): p. 2919-2927.

19. Manor, Y. et al. , *Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian authority*. J Clin Microbiol, 1999. **37**(6): p. 1670-1675.

20. van der Avoort, H. G .A. M. et al. , *Isolation of epidemic poliovirus from sewage during the 1992-3 type 3 outbreak in The Netherlands*. Epidemiol Infect, 1995. **114**(3): p. 481-491.

21. CDC, *Progress toward poliomyelitis eradication - Pakistan, January 2012-September 2013*. MMWR Morb Mortal Wkly Rep, 2013. **62**(46): p. 934-938.

22. WHO, *Wild poliovirus isolated in Switzerland's sewer system; insignificant risk of outbreak* . Euro Polio Page Monthly AFP surveillance bulletin, 2007. **November**.

23. CDC, Update on vaccine-derived polioviruses – Worldwide, July 2012-December 2013.MMWR Morb Mortal Wkly Rep, 2014. **63** (11): p. 242-248.

24. CDC, *Laboratory surveillance for wild and vaccine-derived polioviruses--worldwide, January 2007-June 2008*. MMWR Morb Mortal Wkly Rep, 2008. **57**(35): p. 967-970.

25. Blomqvist, S. et al. , *Characterization of a highly evolved vaccine-derived poliovirus type 3 isolated from sewage in Estonia*. J Virol, 2004. **78**(9): p. 4876-4883.

26. Al-Hello, H. et al. , *Highly divergent type 2 and 3 vaccine-derived polioviruses isolated from sewage in Tallinn, Estonia*. J Virol, 2013. **87**(23): p. 13076-80 LID - 10.1128/JVI.

27. CDC, *Update on vaccine-derived polioviruses--worldwide, July 2009-March 2011*. MMWR Morb Mortal Wkly Rep, 2011. **60**(25): p. 846-850.

28. Roivainen, M. et al. , *Highly divergent neurovirulent vaccine-derived polioviruses of all three serotypes are recurrently detected in Finnish sewage*. Euro Surveill, 2010. **15**(19): p. pii/19566 LID-19566 [pii].

29. Dedepsidis, E. et al. , *Retrospective characterization of a vaccine-derived poliovirus type 1 isolate from sewage in Greece*. Appl Environ Microbiol, 2007. **73**(21): p. 6697-6704.

30. Vinje, J. et al. , *Isolation and characterization of circulating type 1 vaccine-derived poliovirus from sewage and stream waters in Hispaniola*. J Infect Dis, 2004. **189**(7): p. 1168-1175.

31. CDC, *Update on vaccine-derived polioviruses--worldwide, April 2011-June 2012*. MMWR Morb Mortal Wkly Rep, 2012. **61**: p. 741-746.

32. Cernakova, B. et al. , *Isolation of vaccine-derived polioviruses in the Slovak Republic*. Eur J Clin Microbiol Infect Dis, 2005. **24**(6): p. 438-439.

33. Hovi, T. et al. , *Characteristics of an environmentally monitored prolonged type 2 vaccine derived poliovirus shedding episode that stopped without intervention*. PLoS One, 2013. **8**(7): p. e66849 LID-10.1371/journal.pone.

34. Pavlov, D. N. et al. , *Prevalence of vaccine-derived polioviruses in sewage and river water in South Africa*. Water Res, 2005. **39**(14): p. 3309-3319.

35. WHO Global Alert and Response, *Poliovirus detected from environmental samples in Egypt*. 2013.

36. WHO, *Resurgence of wild poliovirus types 1 and 3 in 15 African countries, January 2008-March 2009*. Wkly Epidemiol Rec, 2009. **84**(16): p. 133-140.

37. Hird, T. R. and N. C. Grassly, *Systematic review of mucosal immunity induced by oral and inactivated poliovirus vaccines against virus shedding following oral poliovirus challenge*. PLoS Pathog, 2012. **8**(4): p. e1002599 LID-10.1371/journal.ppat.

38. Ranta, J., T. Hovi and E. Arjas, *Poliovirus surveillance by examining sewage water specimens: studies on detection probability using simulation models*. Risk Anal, 2001. **21**(6): p. 1087-1096.

39. Hovi, T. et al. , *Poliovirus surveillance by examining sewage specimens. Quantitative recovery of virus after introduction into sewerage at remote upstream location*. Epidemiol Infect, 2001. **127**(1): p. 101-106.

40. Lodder, W. J. et al. , *Feasibility of quantitative environmental surveillance in poliovirus eradication strategies*. Appl Environ Microbiol, 2012. **78**(11): p. 3800-5 LID - 10.1128/AEM..

41. Hindiyeh, M. Y. et al. , *Development and validation of a real time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay for investigation of wild poliovirus type 1-South Asian (SOAS) strain reintroduced into Israel, 2013 to 2014*. Euro Surveill, 2014. **19**(7): p. 20710 LID-20710 [pii].

## Appendix 1. Wastewater specimen collection, processing and testing for presence of poliovirus

***Note:*** Wastewater may contain pathogenic microbes and other harmful elements irrespective of its poliovirus content. Laboratory biosafety procedures recommended in the WHO Polio laboratory manual[5] should always be followed. Avoid splashing, and use biological safety cabinet class II in the laboratory for all procedures that risk generating aerosols. When pouring from one vial to another, let the water run on the inner surface of the receiving vial to minimize generation of aerosols. Always use personal protection, and in case of accidental skin contamination, immediately use disinfectant rub if washing with soap and water is not possible.

### *A. Sample collection*

Managers of the National EPI and/or National Polio Programme (NPP), together with senior staff from the Ministry of Health and Sanitary Unit of the Ministry of the Environment are responsible for selecting target populations, based on risk assessments, for environmental surveillance. These populations should be prioritized according to the principles described in these Guidelines. National EPI/NPP managers are responsible for negotiating with local sanitary authorities over access to representative sampling sites, ensuring and details of sample collection are recorded and samples are transported to the processing laboratory. Names and contact details of persons actually doing the collection and arranging the transport of specimens must be known to National EPI/NPP manager.

***Equipment and supplies***

Some sewage treatment plants regularly collect specimens of incoming sewage for their own analysis, sometimes with automated volumetric or time-lapse equipment. This type of sample would generally be optimal for poliovirus surveillance. If this is not feasible, an *ad hoc* grab sampling requires the following materials (to be provided by the NPP if necessary):

- A visually clean and dry ***bucket*** fixed to a shaft or rope to safely reach the stream of wastewater. A pouring lip in the bucket would help pouring the wastewater to the sample vial.
- ***Personal protection materials*** (mask, gloves, apron)
- Sturdy plastic ***sample collection container*** with a volume of 1–2 litres. This should be thoroughly cleaned but sterilization is not necessary. The form of the container is not important (i.e. bottle, can, etc.) but it should be sealable and compatible with the transport container. If containers with small mouth openings are used a clean plastic ***funnel*** may be necessary to avoid splashing.
- Each sample container should be labelled with a sticker marked with an unequivocal and unique ***identification co***de and should be accompanied by an ***information form*** indicating the sampling site, sampling time, and ID of the person collecting the sample. This information should be recorded before filling the container. Use WATERPROOF markers for labelling and do not use paper labels that will disintegrate when they get wet, frozen, etc.
- ***Transport Container*** to be used for cold transportation of one or more samples, labelled with the name and address of the receiving laboratory. It must be sturdy enough to protect

[5] WHO Polio laboratory manual – 4th edition (WHO/IVB/04.10). Available online: http://www.who.int/immunization/documents/WHO_IVB_04.10/en/index.html

the sample containers from compression and equipped with ***cold packs*** capable of keeping the samples refrigerated during the transport.

- ***Zipper bags***, (or other leakage proof plastic bags) one compatible with the sample container, another for the information form.
- ***Gauze, paper towel,*** or other soft absorbent material for sample container support in the transport container and for cleaning potential spills or splashes
- ***Surface disinfectant*** for cleaning the outside of freshly filled sample containers
- ***Disinfectant hand rub*** (or access to soap and clean water) for cleaning potentially contaminated skin
- ***Waterproof marker pen***

***Collection procedure***

- To collect raw wastewater, lower the bucket as closely as is safely possible into the mid-stream of the wastewater. In open canals samples should be collected from below the water surface.
- Fill one or more of the marked sample containers with the collected material, using a funnel if necessary.
- Seal the container(s) tightly, wipe the outer surface with disinfectant, and place the container into the zipper bag.
- Place the wrapped sample container(s) into the transport container, check the presence of ice packs, and add the relevant zipper-bag enclosed information form as well as supporting packing material as appropriate. Seal the container tightly.

***Transport of specimens***

- The packed transport container should be kept at 4°C before and, if possible, also during transport to the laboratory. Poliovirus is inactivated by elevated temperature, so the container should be transported to the processing laboratory as soon as possible.
- Laboratory staff should be contacted, provided with a timetable for sample collection and given the estimated date and time of arrival of the sample in the laboratory.

### *B. Concentration of sewage specimens using the two-phase separation method*

Sewage samples sent cold should be stored at 4$^{o}$C until processing. The concentration will be started immediately after arrival of sample to the processing laboratory and no later than 48 hours of sample receipt.

A mixture of two carbohydrate polymers, dextran and polyethylene glycol (PEG) dissolved in water, will, if left to stand undisturbed for several hours in the cold, separate into two distinct aqueous phases. PEG forms the upper more hydrophobic phase while dextran forms the more hydrophilic, denser, lower phase. Under the correct salt concentrations and pH most viruses likely to be found in sewage samples will concentrate in the lower phase or at the interphase between layers. If the phases are allowed to form in a mixture of sewage sample, dextran and PEG held within a separation funnel, it is possible to collect the lower phase and interphase without disturbing the upper phase. By accurately adjusting the concentrations of the stock solutions and relative volumes of the two polymer solutions it is possible to create a small-volume lower phase, concentrating elements attracted to this phase, including polioviruses and several other enteroviruses.

***Equipment***

- Low-speed *centrifuge*, preferably one with capped swing-out buckets, refrigeration, and a one-round capacity of 500 ml or more
- 2 litre *Erlenmeyr flasks* or a tightly sealable sturdy 1 litre bottles, for each sample to be processed
- *Magnetic stirrer* (+ magnetic bars 6-7 centimers long)
- Heidolph tube shaker or horizontal shaker
- Plexi chamber to cover a shaker and tubes
- Glass beads
- *Separation funnels* with conical bottom, one 1 litre funnel or two 500 ml funnels per sample.
- *Laboratory stand*s with a ring holder or other means to steadily keep the separation funnel in vertical position.
- *Newer model vortex mixer capable of speeds up to approximately 2000 to 2500 rpm.*
- Large *refrigerator* or cold room compatible with the number of stands with separation funnels used at a given time.

***Chemicals***

- Dextran, "Technical quality Dextran T40" from Pharmacosmos, Denmark, (www.pharmacosmos.com) has been widely used; dextran preparations with approximate molecular weight of 40,000 from other manufacturers may be applicable but must first be validated
- Poly(ethylene glycol) (PEG, average molecular weight 6000 (Fluka Analytical; similar preparations from other manufacturers may be applicable but must first be validated)
- Sodium chloride (NaCl, molecular weight 58.44)

***Reagents needed for concentration of four 0.5 litre specimens (to be made in advance!)***

- ***22% (w/w) Dextran***
  - Measure 142 ml sterile distilled water (can be warmed beforehand) into a sterile 0.5 litre laboratory beaker equipped with a sterilized magnetic bar and on a magnetic stirrer
  - Weigh 40g dextran, and add slowly to the water with continuous stirring
  - Continue stirring until a clear, colourless solution is achieved
  - Can be kept for 2 weeks at 4°C.

- ***29%(w/w) PEG 6000***
  - Measure 888 ml sterile distilled water (can be warmed beforehand) into a 1.5 – 2 litre sterile vial equipped with a magnetic bar and on a magnetic stirrer
  - Weigh 363g PEG6000, and add slowly to the water with continuous stirring
  - Continue stirring until a clear, colourless solution is achieved
  - Can be kept for 2 weeks at 4°C.

- Approximately 150 ml ***5N sodium chloride*** (NaCl) solution
  - Fill a 1 litre measuring cylinder or flask with about half a litre sterile distilled water
  - Weigh 5 x 58.44 g = 292.2 g NaCl and add slowly to the water with continuous shaking.
  - Fill with water exactly to the 1 litre mark, transfer all to a larger vial, and mix thoroughly
  - Autoclave (15 min at 115°C) in aliquots, e.g. 100 ml, and store tightly closed at room temperature.

- ***1N sodium hydroxide*** (NaOH) and ***1N hydrochloric acid*** (HCl) for pH adjustment. Dilute from stronger stock solutions.

- **Ethanol-stabilised chloroform**, (bottle may say preservative C2H5OH) which has been stored according to manufacturer's instructions not longer than two years after purchase.

- **pH paper** with 0.5 unit (or tighter) scale

***Concentration procedure of 0.5 litre specimen***

1. Centrifuge approximately 550 ml of the sample, in several portions if necessary, for 20 min at 1500 g (minimum) at 4°C.[6] Pool supernatants in a one litre flask, keep at 4°C. Store the dry pellet at 4°C for later use.
2. Adjust the pH of the combined supernatant to neutral (pH 7 –7.5) using NaOH and HCl. If anything, usually only a few ml 1N NaOH is needed. Pour 500 ml of the supernatant into an Erlenmeyer flask for further processing. Combine the rest with the remaining corresponding raw wastewater sample and keep at 4°C as a backup at least until microscopy of the cell cultures inoculated with the concentrate shows absence of toxicity. After that it will be stored at -20 till all results are ready.
3. To the 500 ml of supernatant, add 39.5 ml of 22% dextran, 287 ml 29% PEG6000, and 35 ml 5N NaCl. Mix thoroughly and keep in constant agitation for 1 hour at (R)oom (T)emperature using a magnetic stir plate at speed sufficient to form a vortex. Note that if RT is high (more than 25°C), the continuous agitation can be done at 4°C.
4. Prepare a sterile conical 1 litre separation funnel (or two 0.5 litre ones) per sample being evaluated and attach the funnel to a stand. Spread grease on the gliding glass surfaces of the valves but do not obstruct the holes. Check water tightness with a small volume of sterile water. Check that the valve is closed tightly. Pour the mixture from #3 into the funnels and leave overnight at 4°C.
5. Observe the lower part of the funnel carefully. Usually a small lower phase and a fuzzy interphase with aggregated solid materials can be seen. Open the valve with caution. Collect the entire lower layer and the interphase slowly drop-wise, into a sterile tube (usually 7–15 ml per 0.5 litre of original sample). It is recommended that when the interphase has been collected, close the valve and disinfect the funnel and upper phase by adding suitable liquid disinfectant and leaving to stand for 30 minutes to 1 hour before pouring all liquids into a container for sterilization and disposal.
6. Resuspend the dry pellet from step #1 with a few ml of the harvested concentrate from step #5 and add it to the rest of the concentrate.
7. Add 0.2 volumes chloroform (e.g. 10 ml of suspension and 2 ml of chloroform). Use only ethanol-stabilised chloroform, (bottle may say preservative $C_2H_5OH$) which has been stored according to manufacturer's instructions not longer than two years after purchase. After addition of chloroform add 1 to 6 grams of sterile glass beads (depends on the amount of concentrate collected), vortex briefly. Shake tubes vigorously for 20 minutes either with Heidolph tube shaker or regular horizontal shaker. The important point is continuous mixing of the concentrate and the chloroform. For security reasons it is recommended that the shaker with tubes is covered by a chamber. Check tightness of tube lids during shaking. Centrifuge according to the WHO Polio Laboratory Manual faecal suspension procedure

[6] g = relative centrifugal force; to convert to RPM use the following formula: $g = 1.118 * r (RPM/1000)^2$

where r = radius in millimetres from centrifuge spindle to extreme point on the tube Hence, here the required RPM in thousands = square root of (1000/1.118*r)

(1500 g* at minimum for 20 minutes at 4°C). Collect the upper water phase in a sterile tube. ***Do not touch the interphase!*** Add antibiotics as for cell culture medium (e.g. penicillin G and streptomycin to final concentrations of 100 IU/ml and 100 µg/ml, respectively).

8. Freeze at least 3 ml aliquot of the extracted concentrate at -20°C (-70°C if available) till all final results are available (at least 6 months). Inoculate 0.5ml aliquots from the remaining extracted concentrate in fresh monolayer cultures of L20B or RD cells in T-25 (25 $cm^2$) flasks. At least 5 LB20 and one RD monolayer flask must be inoculated.

A formula for calculating the volume of original sample being tested for infectivity is following: No. of vials inoculated * 0.5 / volume of concentrate harvested at #7 *starting volume of waste water concentrated. For example, if 500 ml sewage is concentrated to 10 ml and 6 vials are inoculated with the concentrate, the sample volume actually tested is (6 * 0.5 / 10) *500 ml = 150 ml.

### *C. Expected results and problem solving*

Processing of wastewater specimens according to these instructions should result in clearly visible separation of the two phases, usually with a fuzzy interphase. Sometimes, e.g. if the wastewater sample was diluted by abundant rainwater, the material located at the interphase may be minimal but with successful separation, the interphase can be seen due to light reflection.

The nominal virus concentration power of the two-phase separation method is about 50 fold but elements of individual wastewater samples may affect virus recovery. Therefore, quantitative "validation" assessments of accurate virus recovery by spiking experiments are not recommended as a routine, as they may be applicable to the tested sample only. Since NPEV-infections are ubiquitous, and NPEV are also concentrated with this method, it is expected that many if not most wastewater samples reveal NPEV replicating in the RD cell cultures. The expected proportion of NPEV-positive wastewater samples is not a single figure applicable globally but depends on several factors, such as population size, sanitation level, and population density, as well as geographic, climatic and seasonal factors. In addition, the mean wastewater flow per capita of target population at the sampling site, and the proportion of the concentrate inoculated in RD cells affects the likelihood of detecting NPEV. For example, sewage samples representing several hundreds of thousands of urban people in Egypt showed either PV or NPEV at a level close to 100%. In contrast, sewage samples representing a few tens of thousands of an IPV immunized cohort in Indonesia, only partly connected to the sewage network, showed NPEV in less than 10% of cases. Moreover, in Finland, a country using exclusively IPV, increasing the number of inoculated NPEV-susceptible cell cultures from one to six per sample resulted in an increase of the NPEV-positive samples from about 50% to over 90%. In general, provided that the target population size is in the hundreds of thousands or more, the expected percentage of NPEV-positive environmental samples is considerably higher than that of faecal specimens from AFP patients. If there is a suspicion of problems in the processing techniques, contact the GPLN coordinator for technical advice.

An example flowchart for the laboratory testing and reporting of environmental surveillance samples is provided in ***Figure 8***.

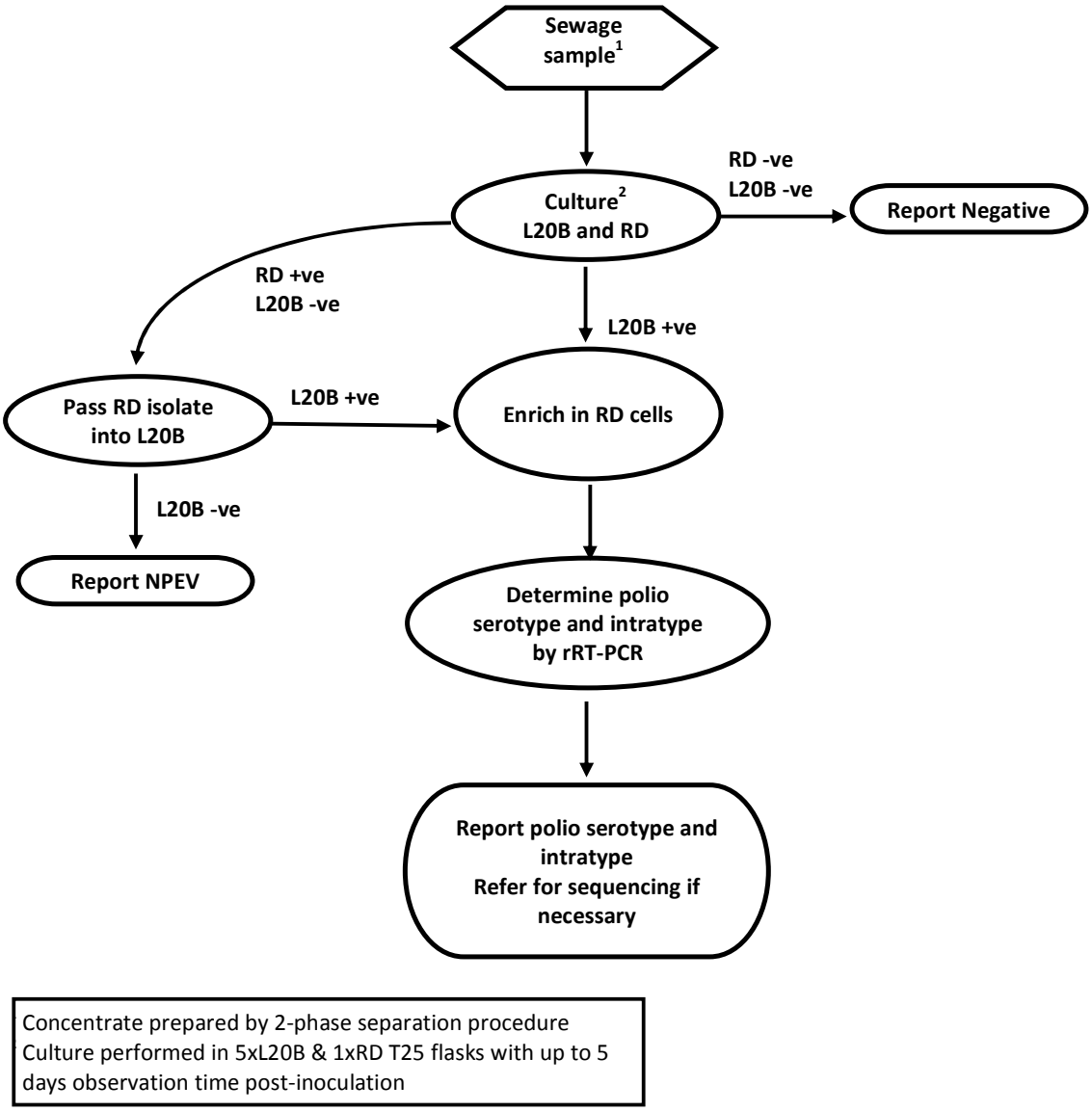


**Figure 8. Flowchart for poliovirus isolation and characterization from sewage samples**

| | |
|---|---|
| Initial volume of raw sewage | 500 ml |
| End Volume (approx.) | around 15 ml |
| Concentration factor | 33 |
| Volume inoculated / flask | 3 ml |
| No of L20B flasks<br>No of RD flasks | 5 flasks (2.5 ml)<br>1 flask (0.5 ml) |
| Proportion Conc. inoculated | 20 % |
| Equivalent in raw sewage | 100 ml |
| Proportion Conc. inoculated in L20B cells | 17 % |
| Equivalent in raw sewage | 85 ml |

**Table 3. Minimal volume of raw sewage to be inoculated into cell cultures**

## Appendix 2. Calculating Theoretical Sensitivity

A mathematical model has been established to help visualize the different factors influencing the proportion of excreted PV that can be recovered at a downstream sampling site, and so estimate the sample and population sensitivities (38). This model demonstrates how the different factors affecting the sensitivity of the surveillance approach are related. For example, how less than optimal virus detection sensitivity could be compensated for by collecting larger volumes for analysis, and for detection of emerging outbreaks of virus circulation, frequently repeated sampling is critical. Applied at the standard bi-weekly analysis of 1 litre of sewage for PV in the Greater Helsinki Region representing about 700,000 inhabitants, the model predicted that an emerging PV circulation would have been detected within a few months or at least as quickly as by using optimal AFP surveillance. This prediction was in reasonable agreement with conclusions drawn from experiments testing the fate of a known amount of attenuated PV flushed into the sewerage system (39). In that trial it was calculated that by analysing a single 400-ml specimen, PV circulation could have been detected if about 100 individuals were infected with PV (2). The recovery of flushed in poliovirus in this trial was surprisingly high, and certainly is variable in different localities because of differences in the composition of raw sewage and in sewerage complexity.

**To calculate a theoretical maximum sample sensitivity** of grab sampling of wastewater from a major collector sewer or inlet of a sewage treatment plant the following assumptions need to be made:

- The mean amount of PV excreted daily per person is $10^7$ infectious units
- All people in the target population are connected to a converging sewer network, and all poliovirus they excrete will end up into the sewer network.
- Once in the sewage, poliovirus will be evenly distributed in the downstream network including the sampling site, and will remain detectable by cell culture for the necessary period of time.
- For this theoretical calculation, the daily flow of sewage at the sampling site, averaged per person of the target population, is taken as 100 litres per person per day. (In true life, it

varies greatly between different localities, and must be known for possible site-specific sensitivity assessment.)

- Recovery of poliovirus through the laboratory procedures is 100%
- The NPL will detect wild poliovirus if there is at least one infectious unit in the aliquots of sample inoculated in cell cultures (1 $CCID_{50}$/1.5 ml, corresponding to the minimum requirement for cell culture).
- Possible coexistence of SL viruses does not interfere with the system.

With these highly optimized assumptions, the following maximal sample sensitivity calculations can be done:

1. **Possibilities to detect a single WPV infected individual.**
   In order to remain detectable without sample concentration the daily amount of virus shed by a single person could be diluted to 15 000 litres of wastewater, with the above optimized assumptions. This volume corresponds to the daily sewage flow representing 150 individuals. If the sample can be concentrated 100-fold, the corresponding source population would be 15 000 people. In practice, the environmental surveillance target populations are usually 10 to 50 -fold larger indicating that the **probability to detect a single poliovirus-infected individual by examining one sewage sample is very small.** It is, however, above zero, and increases by repeated sampling. This is exemplified by the reports describing isolations of VDPVs from sewage samples in several countries (see ***Table 2***). Although the persons shedding the virus have not been identified in these examples, many of the isolates have been genetically characterized as strongly drifted, resembling those found in persistently infected immune deficient persons. Typically, only a proportion of successive samples at a given sampling site have been VDPV-positive reflecting low overall concentration of the virus, intermittent shedding, or both.

2. **Possibility to detect poliovirus transmission in the target population**
   During poliovirus transmission in a fully susceptible population living in close contacts with each other, most of the infected individuals may be close to the peak phase of virus shedding, and the above calculations can be used to predict that the virus could be in one sample if one out of 15,000 individuals (or about 0.01%) is shedding. However, in partially immune populations virus transmission is not explosive, and most of the infected individuals at any given time are well beyond the peak phase of daily virus output. Using a mean daily output of $10^5$ infectious units per person in the calculation, the required proportion of infected individuals in the population would be about 1% to be detected in a given sample. Repeated sampling, for example on a weekly basis, will increase the probability of detecting ongoing transmission.

**Note:** These calculations are based on the highly optimized assumptions listed above. In reality, with several non-optimal variables in the system, the sample sensitivity is much lower. Cross-sectional screening of environmental samples from different locations may lead to ***false negative results***. Long term monitoring of carefully selected sampling sites by repeated sampling is likely to give much more reliable information on the presence or absence of poliovirus transmission.

## Appendix 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 programme response, including:

- the status of the country as polio-free, recently endemic or endemic;
- the polio immunization coverage in the population;
- the quality of AFP surveillance in the population;
- the specific goal of environmental surveillance;
- rank of the isolate: the very first or repeated observation.

In recently or currently polio-endemic areas, WPV or VDPV detected in the environment serve 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 environmental surveillance strategies represent a public health emergency warranting 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, as discussed in ***Appendix 2*** on maximal theoretical sensitivity of environmental surveillance, this should be considered as extremely rare. In a well-immunized population detection of a WPV or VDPV may be determined not to pose a risk of starting transmission. However, if virus is detected through environmental surveillance **epidemiological and further laboratory 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. Inform WHO within 48 hours that a suspected outbreak is being investigated.

2. Enhanced 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 sensitivity of virus detection. Ongoing transmission may be deduced from repeated wild poliovirus detection through intensified sampling (e.g. weekly sampling).
    - 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 (non-polio AFP 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 routine 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.
   - Take into account the type of vaccine used (OPV, IPV or both).

5. Enhance virological investigations
   - Expedite genome characterization of the wild poliovirus or VDPV isolates to assist in the investigation of their possible source and possible chains of transmission.
   - To increase population sensitivity request that all virus-negative faecal specimens collected during the same time period and untyped or non-typable virus isolates from faecal and environmental samples be submitted to a WHO-accredited laboratory for further investigations.
   - "Flag" all subsequent poliovirus isolates, environmental samples and faecal samples 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. An outbreak is confirmed if ***any*** of the following conditions are met:

- Detection of WPV or VDPV from two or more independent environmental samples (e.g. different sampling sites or on different days within a few weeks);
- evidence of genetically distinguishable wild polioviruses or VDPV (i.e. $\geqslant$ 2 nucleotides difference in VP1 region) in a same environmental sample; or
- paralytic polio cases, polio-compatible cases, wild poliovirus or VDPV-infected persons are found during follow-up investigations.

**Note:**

***a. Separate introductions cannot be ruled out if the number of nucleotide differences in the viral capsid protein 1 genes is significantly greater than expected for person to person transmission during the time interval between isolations and if each isolate has a closer similarity to viruses isolated outside of the country or region than the isolates have to each other.***

b. ***If a VDPV strain in genetic characterization is found to resemble iVDPV, repeated isolation of the same or a genetically distinct but related virus from the same site does not necessarily indicate an emerging outbreak. The regional GPLN coordinator should be contacted immediately if VDPV is suspected.***

If an outbreak is confirmed, countries should notify 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, consistent with current WHO guidelines on outbreak response[7], and include the following actions:

- Contact all surveillance units and major hospitals nationally to inform them of the outbreak and provide them with information and materials to assist in identifying further cases. Enhance national surveillance activities to determine whether poliomyelitis cases occurred in areas beyond that where the outbreak was confirmed.
- Institute systematic monitoring of surveillance reports at the national level.
- Conduct an immunization response consistent in size and nature with the findings of the outbreak investigation.

[7] Responding to a polio outbreak . GUIDELINE . 7 January 2011. Available online from: http://www.polioeradication.org/Portals/0/Document/Resources/PolioEradicators/1a.PolioOutbreakGuideline20110107.pdf

## Appendix 4. Example Standard Operating Procedure for sample collection

**Standard Operating Procedure (SOP) For Wastewater Sample Collection for Environmental Surveillance**

SOP No: ENV-SUR Ver. 01

Purpose: *Collection of Wastewater Samples from Designated Sites in Karachi*

Author: Dr. ---

Reviewed by: Dr. ----

Reference: Guidelines for Environmental Surveillance of Poliovirus Circulation (WHO)

Approved by: Dr. ----

### Equipment

1. Plastic Jerrycan (new), 1-1.5 liters
2. 5 liters bucket
3. Approximately 7 meter rope
4. Gloves (thick/large size)
5. Masks
6. White gown/apron
7. Zipper bags (Large and small)
8. Permanent Marker
9. Liquid bleach
10. Gauze
11. Sealing Parafilm tape
12. Atmospheric measuring thermometer
13. Large sticker or plaster (to be fixed on the Jerrycan to write details)
14. Sample transportation box/Carrier with 5 ice packs and zipper envelop for lab request form.

**Collection of grab wastewater samples**

1. Local municipal/health authority should be informed of schedule of the sample collection.
2. Always arrange all the equipment/logistics required one day before collection of sample.
3. Sample should be collected at 8 to 9 a.m.
4. All protective measures should be adopted before collection of samples.
5. Avoid taking samples during rainstorms for personal safety reasons, to protect surveillance equipment, and because rain may enter the sewage system and dilute virus to levels below detection thresholds.
6. Lab request form should be filled just before collection of sample.
7. Atmospheric temperature should be noted just before collection of sample.
8. Wear the gown/apron before sample collection (use mask if required).
9. Sample should be collected from mid-stream of inlet to the drainage Nulla/Canal/Nadi/River by bucket.(sample should only be collected from running stream)
10. Collect 1 to 1.5 litter of wastewater in Jerrycan.
11. Close and seal the Jerrycan properly with Parafilm tape to ensure no leakage, clean Jerrycan from outside by liquid bleach with the help of gauze and let it dry.
12. Mark the Jerrycan with permanent marker or paste the sticker / label with all the details of sample on it.
13. Place the Jerrycan filled with wastewater in zipper bag properly (keeping no air inside zipper bag while closing the zip) and then in the transportation box/sample carrier, keeping the ice packs on all sides of Jerrycan.
14. Sterilize the bucket with liquid bleach and let it dry.
15. Place the duly filled Lab. request form in a small zipper plastic envelop and fix it on side of transportation box/carrier.
16. Shift the transportation box/carrier to MOH/WHO office, to make arrangement to send it to the laboratory, , through courier service immediately (if not possible, keep the sample in refrigerator at 2 to 8 C, to send it on next available flight).

17. Always place fresh well frozen ice packs inside the transportation box/carrier, before handing over to courier service.
18. Keep the follow up with the laboratory for the quality of sample and results.

## Appendix 5. Evaluating environmental surveillance systems.

Changes in the polio risk status or the ecological or demographic characteristics of a location may make a site more or less relevant to the polio surveillance programme. Sites should be reviewed by WHO regional offices and country programmes on a reasonably frequent basis to determine if they are providing appropriate high-quality and relevant information. Factors to be considered when attempting to evaluate the performance and determine the added value of existing environmental surveillance systems include:

1) Target population: appropriateness/representativeness of estimated population in catchment of sampling area(s)
   - Vaccination status of the population
   - Likelihood for poliovirus circulation based on current and historic epidemiology
   - Sanitation in community of interest
   - Population density
   - Mobility of targeted population

2) Sampling adequacy: adequacy of sampling to detect polioviruses
   - Structure of the wastewater disposal systems
   - Sampling points in relation to mixing of water, waste, flow of water
   - Exposure to industrial waste water containing components likely to accelerate inactivation of PV or be toxic to cell culture systems.
   - Frequency, time and size of sampling

3) Field operational and performance factors: ability of field personnel to ensure proper sampling and transportation of environmental specimens
   - Proper planning, implementation and monitoring of the surveillance system
   - Ease of transport and logistics for sending environmental samples to an accredited polio laboratory

4) Laboratory operational and performance factors: ability of laboratory to detect polioviruses
   - Presence/absence of a polio laboratory within the country
   - Historic and current capacity of the laboratory to isolate polioviruses using recommended methodologies.
   - Laboratory capacity to perform additional procedures needed for environmental samples
   - Laboratory space, equipment and personnel
   - Availability of sustained funding for laboratory activities