Summary report on the

Twenty-first intercountry meeting for directors of poliovirus laboratories in the WHO Eastern Mediterranean Region

WHO-EM/POL/441/E

Muscat, Oman
22–24 January 2020

World Health Organization
REGIONAL OFFICE FOR THE Eastern Mediterranean

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1. Introduction

The twenty-first intercountry meeting for directors of poliovirus laboratories in the World Health Organization (WHO) Eastern Mediterranean Region was held by the WHO Regional Office for the Eastern Mediterranean in Muscat, Oman, on 22–24 January 2020. The meeting was attended by the directors of poliovirus laboratories from Egypt, Iran (Islamic Republic of), Iraq, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Syrian Arab Republic, Sudan and Tunisia. Participants also included experts from: Centers for Disease Control and Prevention (CDC), United States of America; National Institute for Biological Standards and Control (NIBSC), United Kingdom; National Institute of Public Health and the Environment (RIVM), Netherlands; Kenya Medical Research Institute (KEMRI), Kenya; and the National Virology Laboratory, Zimbabwe. Staff from WHO headquarters and the Regional Office, and an independent consultant from Finland, also attended.

Dr Humayun Asghar, Coordinator, Polio Eradication, WHO Regional Office, and coordinator of the regional poliovirus laboratory network, welcomed participants, and a minute's silence was observed for the passing of His Majesty, the late Sultan Qaboos bin Said.

Dr Hamid Jafari, Director, Polio Eradication, WHO Regional Office, delivered a message on behalf of Dr Ahmed Al-Mandhari, WHO Regional Director for the Eastern Mediterranean, commending the excellent work performed by the regional polio laboratory network, particularly the achievements of the network's laboratories in implementing new methods, techniques and developments in poliovirus diagnosis. Following Dr Al-Mandhari's message, Dr Seif Al Abri, Director-General for Disease Surveillance and Control at the Omani Ministry of Health, welcomed participants and expressed the commitment of Oman to eradicating polio.

The specific objectives of the meeting were to:

- review the regional polio laboratory network performance;
- provide technical information on issues related to the global polio eradication initiative;
- discuss the role of poliovirus laboratories in the polio endgame strategy, Global Action Plan III (GAPIII) phase 1 activities and environmental surveillance; and
- develop recommendations for further improvement in laboratory performance.

2. Summary of discussions

Global Polio Eradication Initiative and Global Polio Laboratory Network

WHO global and regional polio laboratory coordinators gave presentations on the current state of the Global Polio Eradication Initiative (GPEI) and an overview of the performance, activities and challenges faced by the WHO Global Polio Laboratory Network (GPLN) and planned endgame strategies. They congratulated laboratory personnel on achieving the landmark certification of wild poliovirus type 3 (WPV3) eradication and expressed appreciation for their commitment to eradicating wild poliovirus type 1 (WPV1). They noted that the increasing number of circulating vaccine-derived poliovirus type 2 (cVDPV2) outbreaks since the switch from trivalent oral polio vaccine (tOPV) to bivalent oral polio vaccine (bOPV) in 2016 had led to a corresponding increase in GPLN workload. They highlighted that the ongoing cVDPV2 outbreaks must be controlled by implementing the new strategies that are being developed. The achievements of polio laboratories in the Region, including the efficient and high quality work of laboratories for both acute flaccid paralysis (AFP) and environmental surveillance, and the establishment of new laboratories able to conduct intratypic differentiation (ITD) of

polioviruses by real time polymerase chain reaction (PCR) in Jordan and Morocco, were commended.

Key aspects of the regional network's laboratory quality assurance programme were discussed, with a focus on biosafety related to the handling of poliovirus. All 16 elements of the biorisk management system described in Annex 6 of the WHO global action plan (GAPIII) will be assessed for all laboratories in the Region using a tool provided by WHO. Performance in the Region continues to be excellent, with laboratories providing high quality information on poliovirus isolation and characterization in a timely manner, which is essential for guiding GPEI activities. Laboratories in the Region are also actively engaged in supporting endgame activities such as pilot testing of improved laboratory methods.

Status of polio eradication in the Region

Two countries remain endemic for polio in the Region, with continuation of indigenous WPV1 transmission. Furthermore, the polio eradication programme in the Region faces particular challenges, including security problems, a lack of access to children, threats to the lives of polio workers and continuing conflict and political turmoil. Despite the enormous challenges and obstacles, significant progress has been made thanks to the efforts of thousands of personnel and vaccinators. In 2018, only 33 polio cases were identified in two countries (21 in Afghanistan and 12 in Pakistan), all due to WPV1.

However, in 2019 Pakistan experienced a WPV1 outbreak of 138 cases and 28 cases were reported in Afghanistan. The genetic heterogeneity among isolates from Pakistan appears to have decreased between 2018 and 2019, with the number of different genetic clusters in which AFP polio isolates are classified dropping from eight in 2014 to four in 2019.

But these foci of endemic circulation in both Pakistan and Afghanistan continue to delay global polio eradication and pose a risk of international spread, particularly to countries at risk due to conflict and expanding areas of inaccessibility. The completion of poliovirus eradication in these two endemic countries remains jeopardized by the lack of safe access to children in conflict-affected areas and inconsistent improvement in the quality and coverage of supplementary immunization activities in key areas. In addition, as of January 2020, 15 cVDPV2 cases had been reported in Pakistan.

Polio laboratory network performance

Performance of the regional polio laboratory network remains high despite the numerous difficulties and challenges. All regional polio network laboratories are fully accredited and maintain certification-standard performance indicators, efficiently supporting global polio eradication activities. WPV1 and vaccine-derived polioviruses continue to be detected with speed and accuracy despite the rise in workload due to improved AFP surveillance and increased sampling from contacts of AFP cases in infected districts. Moreover, some laboratories, such as those in Iraq and Syrian Arab Republic, are performing under challenging security situations.

The VACSERA laboratory in Egypt has been an excellent performer for several years, acting as a reference laboratory and providing virus isolation, ITD and nucleotide sequencing for Iraq, Lebanon, Sudan, Syrian Arab Republic and Yemen. Environmental surveillance was introduced in in Egypt in 2001 and is well established, with collection sites covering all 27 governorates.

The support of the Kenya Medical Research Institute (KEMRI) poliovirus laboratory to countries of the Region is highly appreciated.

The laboratory serves Comoros, Eritrea (up to 2018) and Kenya in the WHO African Region and Djibouti and Somalia in the Eastern Mediterranean Region. The laboratory also receives samples from Yemen as well as samples from cross-border cases. The laboratory supports both AFP and environmental surveillance in Kenya and Somalia.

The performance of the KEMRI laboratory has been good over the years, and all performance indicators on sample numbers, timeliness and accuracy, proficiency testing and onsite reviews have been consistently good since 2007. The laboratory coped with a substantial increase in the workload during 2019, due to the testing of samples received from Yemen, in addition to Djibouti and Somalia. The laboratory is being renovated, which poses a major risk to samples being tested on time. However, the renovation will be completed in March 2020.

Virus surveillance

The WHO regional reference laboratory in Pakistan continued to maintain its high-level performance during 2019. Laboratory quality indicators, such as cell culture results reported within 14 days or ITD results reported within seven days, were well above the minimum requirements for both Afghanistan and Pakistan. Stool samples for 29 054 AFP cases and 5196 contacts were received from Pakistan during 2019, and a total of 138 WPV1 and 15 cVDPV2 cases identified. Stool samples for 7425 AFP cases and 1031 contacts were received from Afghanistan, with 28 WPV1 cases identified. The non-polio enterovirus isolation rate was 17% and 16% for Pakistan and Afghanistan, respectively. Additionally, 828 environmental samples from 60 different sites in Pakistan were analysed. Of these, 360 samples were found to contain WPV1 strains and 25 were positive for cVDPV2 isolates. Environmental surveillance in Afghanistan was initiated at the end of 2013 at three different collection sites, and increased to 21 sites

in 2019. Of the 287 environmental sewage samples received from these sites during 2019, 52 samples were positive for WPV1 and no vaccine-derived polioviruses were isolated.

The National Virology Laboratory of Zimbabwe also participated in the meeting. During, 2019, they received and processed stool samples from 691 AFP cases, 331 contacts and 223 healthy children in Yemen. Among these samples, only eight Sabin-like (SL) polioviruses (three SL1 and five SL3) were isolated. The isolation rate of non-polio enterovirus was recorded at 6%.

Molecular epidemiology

All WPV1 isolates from Afghanistan and Pakistan belong to the South Asia (SOAS) genotype. All cases were detected from the Northern Corridor (Kabul, Nangarhar, Kunar, Peshawar, Lakki Marwat, Bannu, and North and South Waziristan), Southern Corridor (Pishin, Quetta, Killa Abdullah, Helmind and Kandhar) and Karachi zones, showing persistent circulation of WPV1. The genetic heterogeneity among isolates from Afghanistan and Pakistan appears to have decreased slightly in 2019 as viruses from four genetic clusters (groups of polioviruses sharing $\geq 95\%$ sequence identity in the VP1 viral capsid protein) were detected in Pakistan, compared to five genetic clusters in 2018. Environmental surveillance data clearly demonstrates that in several areas of Pakistan, including Baluchistan, Punjab and Islamabad, Sindh and Peshawar, WPV continues to circulate in the absence of reported AFP cases, and that significant susceptible populations exist in these areas.

Similarly, in Afghanistan, a significant drop in terms of genetic diversity occurred during 2019 and the number of genetic clusters decreased to two compared to 2018 when there were four distinct genetic clusters.

Population migration is still regarded as the major source for the transmission of WPVs between different areas in both countries. The core reservoirs have remained active, including southern (Helmand) and eastern Afghanistan (Nangarhar), with persistent WPV1 circulation that needs urgent attention. Core reservoirs in Pakistan, including in Khyber-Peshawar, Quetta block and Karachi, have also remained active and transmission within these reservoirs remains sufficiently intense, as is evident by sustained local circulation and continuous reseeding of non-reservoir areas, such as in parts of Punjab, northern Sind and Baluchistan. Unfortunately, WPV1 transmission was re-established in Lahore (Punjab) due to importation of virus from Bajour (Khyber Pakhtunkhwa) in 2019.

Currently, the most intense circulation is in southern Khyber Pakhtunkhwa where an outbreak centred on Lakki Marwat and Bannu is seeding virus across Pakistan and parts of Afghanistan. The Quetta block has been re-infected by two-way importation (Karachi–Kandahar). Migrant population groups pose a high risk to successful eradication of polio in the one epidemiological block if not identified and reached.

The continuing detection of orphan viruses from both countries, with a divergence of between 1.5% and 2.3% from closest known isolates, demonstrates that viruses can circulate undetected for periods of more than 1.5 years in the community. These findings also indicate gaps in the surveillance system that urgently need to be filled if eradication is to be achieved.

Vaccine-derived polioviruses

The cVDPV2 outbreak in Somalia (emergence group SOM-BAN-1) was first detected in an environmental sample collected in Banadeer in October 2017. This emergence was widely detected throughout Somalia

in Banadeer, Hiran, Lower Juba, Gedo, Togdher and Bari provinces, as well as in Ethiopia and Kenya. Since first being detected, 13 cases and 24 environmental samples (mostly from Somalia) have been further identified related to this emergence (latest detection in December 2019). Genetic sequencing has shown that viruses from this emergence are highly divergent from the parental Sabin 2 (36–53 nucleotide substitutions) and thus the geographical origin of the emergence cannot be definitively resolved. All SOM-BAN-1 cVDPV2 had reverted at the 5' UTR and VP1 attenuating sites and the non-capsid sequences at the P2 and P3 regions showed distinct recombinant genomes (vaccine/vaccine and vaccine/nonvaccine recombinants). Phylogenetic analysis assuming a constant rate (molecular clock) of evolution of 1.1% per year, resulted in an estimated date of oral poliovirus vaccine type-2 (OPV2) origin of March 2014, indicating that the originating OPV2 was likely before the April 2016 tOPV to bOPV switch. There was no monovalent OPV2 (mOPV2) use before the targeted mOPV2 campaigns that started in 2018 for outbreak response. Lower Juba is the most likely area of persistent cVDPV2 transmission in Somalia, with periodic detection in environmental surveillance that is currently restricted to the Banadeer region.

The cVDPV3 outbreak in Somalia (SOM-BAN-2) was first detected in February 2018 and the latest detection was September 2018. SOM-BAN-2 was detected only in Somalia in Banadeer, Lower Juba, Middle Shabelle and Hiran provinces. Genetic sequencing showed that cVDPV3 were moderately divergent from parental Sabin 3 (14–23 nucleotide substitution differences from Sabin 3) and molecular clock analysis estimated the date of oral poliovirus vaccine type-3 (OPV3) origin in mid-2016.

Since April 2019, cVDPV2s have been detected in several provinces of Pakistan (Gilgit Baltistan, Khyber Pakhtunkhwa, Punjab, Sindh and

Islamabad) from AFP cases and environmental samples. Genetic characterization of the VP1 region showed that newly-detected cVDPV2s in 2019 were not genetically related to previously known cVDPV2s (last detected in 2016). All 2019 cVDPV2s showed limited divergence from parental Sabin 2 sequence (6–15 nucleotide differences). Ongoing VP1 analysis shows evidence of genetic relationship among cVDPV2s which indicates multiple cVDPV2 emergences. Three cVDPV2 emergences were first detected in Gilgit Baltistan and two cVDPV2 emergences were first detected in distinct districts of Khyber Pakhtunkhwa. Ongoing analysis of complete genomes provides support to initial characterization of cVDPV2 isolates using VP1 sequences. The limited extent of divergence of cVDPV2 isolates strongly suggests that the date of originating OPV2 is well after the tOPV to bOPV switch in April 2016. The origin of these outbreaks is unknown as no mOPV2 vaccine has been used in Pakistan since 2017.

Primary immunodeficiency disorder (PID) surveillance and immunodeficiency-related vaccine-derived poliovirus (iVDPV)

PID and iVDPV surveillance has been established in Egypt, Islamic Republic of Iran and Tunisia as a regional pilot project for the integration of PID in AFP surveillance.

Tunisia is the first country in the Region to have systematically integrated PID into the national AFP surveillance programme. The Institut Pasteur de Tunis, a regional reference laboratory, in collaboration with the national PID programme, has integrated PID surveillance with AFP surveillance to detect any excretion of poliovirus in children diagnosed with immune deficiency. Studies on poliovirus excretion in PID patients were conducted in Tunisia in 1997, 2009–2010 and 2014. Since 2015, the laboratory has received a few requests for enterovirus detection in PID patients as part of its routine diagnostic

activities in clinical virology. Two iVDPV3 cases have also been detected through AFP surveillance. Case 1 (6 months of age) was detected in November 2016. There was no history of immunodeficiency in the family. The patient had no history of repetitive infections or any symptoms of immunodeficiency. Virological investigation in stool 1 and stool 2 revealed pre-VDPV3 isolates with 6 and 8 nucleotide changes in VP1, respectively. Immunological investigation confirmed major histocompatibility complex (MHC) class II deficiency. The virus isolated in follow-up stool samples at D21 and D43 confirmed as iVDPV3 with 10 and 11 nucleotide changes, respectively. The patient died in February 2017. Patient 2 (9 months of age), brother of patient 1, was diagnosed with AFP in March 2019. Virological investigation in stool 1 and stool 2 revealed VDPV3 with 13 and 15 nucleotide changes in VP1, respectively. Immunological investigation confirmed MHC class II deficiency. The patient is still alive with residual paralyses and is being followed for virus excretion. Sample 10 collected on 9 December 2019 (D271) revealed 37 nucleotide changes in VP1.

The National Polio Laboratory in Islamic Republic of Iran started screening PIDs for poliovirus excretion in 2009, when the pilot project was put in place. In 2013, the iVDPV project was approved by the Polio Research Committee and 291 PID cases were screened for poliovirus excretion up to January 2020. The first detected case of iVDPV in the country was in 1995 from an AFP case with antibody deficiency. Since then, 26 iVDPVs have been detected: 19 from AFP cases and seven from patients with primary humoral immune deficiency. Among the 26 cases from whom iVDPVs were isolated, four are chronic excretors.

In Egypt, the VACSERA laboratory reported four iVDPV cases (one iVDPV1 and three iVDPV3) in 2019. However, from 2011 to 2018 the laboratory tested 297 PID patients and confirmed 13 cases as iVDPVs. All patients stopped iVDPV excretion after treatment with intravenous

immunoglobin. The project is being continued and expanded to better understand the risks iVDPV excreters pose to global poliovirus eradication.

Environmental surveillance

The current sewage concentration method recommended by the GPLN for environmental surveillance is the two-phase separation method. Other methods are described in the literature, and laboratories of the GPLN are actively testing different techniques with a view to completing WHO guidelines that will include detailed standard methods and accreditation procedures to ensure quality assurance. Afghanistan, Egypt, Islamic Republic of Iran, Jordan, Kuwait, Lebanon, Pakistan, Syrian Arab Republic and Sudan have implemented environmental surveillance to detect polioviruses in sewage water in strategic locations.

In Pakistan, there are 60 active sewage wastewater collection sites. Environmental surveillance has enabled the programme to more promptly assess and rapidly respond to new circulations/outbreaks. In response to emerging cVDPV2 outbreaks, 12 ad-hoc sites have been opened since April 2019, with fortnightly sampling. In addition to this, fortnightly sampling is being carried out from nine routine sites in Pakistan and six routine sites in Afghanistan, which has considerably increased the workload of the laboratory. A bag mediated filtration system study has been carried out in 12 sites since February 2016. A total of 828 samples were tested during 2019, with 43.5% found to contain WPV1 isolates and 3% containing cVDPV2 isolates.

In Afghanistan, there are 21 active sites in 19 districts/towns. A total of 260 samples were tested during 2019, and 21.1% of them were shown to contain WPV1 isolates.

Since the establishment of environmental surveillance in Islamic Republic of Iran, 128 sewage samples have been analysed, of which most are positive for SL viruses, 16 were positive for non-polio enterovirus only and five were negative. In April–May 2019, three WPV1 were isolated in sewage samples from Konarak, Sistan and Baluchestan Province. Complete VP1 sequencing was performed at the Iranian national polio laboratory. The sequencing data was sent to the Pakistan laboratory for further investigation. A phylogenetic tree was constructed using the neighbour-joining method based on the Kimura two-parameter model. Phylogenetic analysis confirmed that the wild polioviruses isolated from Konarak were closely related to WPV1 circulating in Karachi, Pakistan. Follow-up samples were negative for WPV1 and the event was closed in January 2020.

Environmental surveillance in the Syrian Arab Republic has been expanded to 16 sites covering all governorates, except Idlib. During 2019, a total of 183 environmental samples were processed and no WPV1 isolated. Moreover, ITD/VDPV real-time reverse transcription PCR (rRT-PCR) was started in May 2018 after the training of laboratory staff. Similarly, environmental surveillance is continuing in Jordan, Sudan and Lebanon where three, four and five sites were operational, respectively, in 2019. No WPV1 was isolated from these sites during 2017–2019.

During 2019, the KEMRI laboratory received 296 samples collected from 17 different environmental sites in Kenya. The laboratory also supports poliovirus detection in environmental samples from Somalia, including from Urobo 2, Kawma and the Egyptian hospital in Banadir, Mogadishu. In 2019, the laboratory collected 63 samples from these sites and reported five cVDVP2, with the last date of sample collection being 22 December 2019.

There was discussion on plans to expand environmental surveillance to Libya, Oman, Qatar, Saudi Arabia and United Arab Emirates where high risk populations reside.

Laboratory quality assurance

Annual proficiency testing and assessment of laboratories continues to be critical for quality assurance of the performance of polio laboratories. Four different proficiency testing panels are in use: accuracy of virus isolation; ITD by rRT-PCR; rRT-PCR for VDPV screening; and sequencing poliovirus isolates. The proficiency testing programme is coordinated by WHO in collaboration with specialized laboratories in the United States of America and the Netherlands. A standardized test to measure the sensitivity of cell lines for poliovirus infection is also required for laboratories to be accredited.

At the time of the meeting, all laboratories had passed the most recent proficiency tests for ITD algorithm, except two that were waiting for a proficiency testing panel. Similarly, three laboratories in the Region (in Egypt, Pakistan and Tunisia) are doing nucleotide sequencing and passed proficiency testing panels in 2019 with a 100% score. Regional laboratories that participated in virus isolation proficiency testing achieved scores of 95–100%. A few laboratories have not been able to be virus isolation proficiency tested yet due to transportation issues.

Previously, there have been issues with the interpretation of rRT-PCR ITD/VDPV data throughout the regional laboratory network, but the sharing of ITD rRT-PCR practice files by the Pakistani laboratory with all regional laboratories has improved the performance of laboratory personnel in data interpretation and reporting.

Overall, regional laboratories are highly proficient in polio PCR assays, the turnaround time is excellent for all laboratories and proficiency test results correlate with routine results.

Poliovirus laboratory contingency planning

There was discussion on the need to devise contingency plans to respond to unforeseen increases in workload due to a polio outbreak, equipment failure and/or non-polio emergencies requiring the use of resources from the polio laboratory.

A detailed polio laboratory contingency plan was described by the Omani national poliovirus laboratory. This was first drafted in 2017 based on the recommendation of the regional polio laboratory directors meeting. The objective of the plan is to mitigate the risk of system breakdown and unacceptable service unavailability in the case of a crisis or outbreaks, and ensure that the laboratory can operate effectively and without excessive interruption or delay during an outbreak or other disruption of normal functioning of the laboratory. While the plan is mainly focused on WPV/cVDPV outbreak situations, it can also be activated in other situations that disrupt laboratory functioning. Furthermore, the plan allows a laboratory to guarantee that the necessary quality standards will be met during a crisis situation and serves as a reference manual for all laboratory personnel in case of a contingency.

The importance of risk assessment was highlighted. The risk to individuals and the community has been minimized in the plan by use of recommended mitigation measures, such as the use of proper personal protective equipment, provision of containment devices and proper infectious waste management. The plan is part of the national polio importation planning. Operational areas for contingency planning were also discussed, including the space, storage area, power supply,

data backup, supplies and stockpiling, equipment, inventory, human resources, staffing, training, communication and coordination. The plan should be reviewed and updated regularly.

Simulation exercises

During the meeting all participants were given the opportunity to participate in simulation exercises to prepare for different emergency situations that laboratories may face in the post-eradication era. A scenario of a poliovirus outbreak in a polio-free country was given to all participants, who were asked to assess the risk. This exercise was liked by the participants who requested more of such exercises.

Similarly, a simulation exercise has been regularly conducted for many years on ITD/VDPV rRT-PCR data interpretation and reporting. This exercise is performed on the ABI 7500 run files (.eds) shared by Pakistan's regional reference laboratory with other laboratories in the Region that have the same instrument. The purpose is to improve capacity-building and prepare low workload laboratory staff for poliovirus PCR data interpretation and reporting. This exercise will be extended to those laboratories who have other real time PCR thermocyclers, such as Rotor-Gene, in coordination with NIBSC. During the meeting the performance of regional laboratories was monitored for this exercise.

3. Recommendations

1. The Regional Coordinator, in collaboration with WHO headquarters, should work towards establishment of a laboratory surge hub to support laboratories to:
   - identify and overcome limitations affecting optimal performance, such as in logistics, resources and training needs;
   - facilitate quick responses to increasing/changing testing demands; and
   - facilitate additional refresher training and training on implementation of new methods.
2. In a world of changing public health demands, the heads of laboratories, in collaboration with ministries of health and national authorities/stakeholders, should work with WHO to ensure the use of national resources to maintain the highest level of polio surveillance and respond to the increasing testing demands required for the completion of global polio eradication. This includes:
   - the identification of alternative resources for sustaining laboratory functions at certification standards to address the potential reduction in GPEI resources; and
   - working with national vaccine preventable disease surveillance and health emergency units to prioritize integration of existing polio assets in other relevant public health priority areas.
3. Each laboratory should complete a comprehensive contingency plan to address the challenges in outbreak or emergency settings placed on testing demand and submit it to the Regional Coordinator for review. In addition:
   - the plan should be approved by the competent authority at the institutional and ministry level; and
   - should be activated in consultation with the ministry of health in outbreak/emergency situations.

4. Laboratories should submit annual reports on laboratory performance through the global polio laboratory network management system before 15 February each year.
5. Laboratory staff should contribute to regular monitoring/review of all environmental surveillance sites for sensitivity, functionality and country relevance, using recommended WHO environmental surveillance laboratory performance indicators. Expansion of environmental surveillance sites should be rationalized using epidemiological data and an assessment of the workload and human and laboratory resources needed to maintain efficiency without compromising the quality of laboratory work.
6. All laboratories that have a low workload of rRT-ITD PCR assays should run at least one ITD/VDPV reaction per month in order to maintain competency and quality assessment of ITD equipment/reagents.
7. All isolates from PID patients with an SL result on ITD rRT-PCR should be referred for VP1 nucleotide sequencing.
8. Laboratories should be informed of the possible consequences associated with implementation of the new assays for direct detection and identification of poliovirus from stool samples, so that they are able to fully assess the impact on laboratory capacity, infrastructure, equipment, human resources needs and so on, and can make any necessary adjustments before implementation. Accordingly:
    - WHO in collaboration with CDC should plan training for laboratories for implementation of the new direct detection assays; and
    - laboratories should be aware of use of novel OPV2 (nOPV2) strains. A detailed algorithm for detection of nOPV2 strains should be shared with laboratories as soon as it is finalized and approved by the WHO Small Working Group.

9. Polio laboratories and laboratory coordinators should contribute to the development of a stepwise and systematic process for the analysis of nucleotide sequencing data to make key information more accessible to polio surveillance officers and help improve understanding of the epidemiological history and significance of any poliovirus isolate identified in the laboratory. The need for training in this area should be discussed with the Regional Coordinator.
10. All laboratories should complete an assessment of all 16 elements of the biorisk management system described in Annex 6 of GAPIII using the regional Excel tool provided by WHO. A mitigation plan should be developed based on the gaps and deficiencies found in the risk assessment and shared with the Regional Coordinator.
11. Simulation exercises for different emergency scenarios faced in laboratory should be conducted for staff training. RIVM will help to design these exercises.

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