

Summary report on the

Seventeenth intercountry meeting of directors of national and regional polio reference laboratories in the Eastern Mediterranean Region

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Amman, Jordan
26–28 January 2015



**World Health
Organization**

Regional Office for the Eastern Mediterranean

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1. Introduction

The seventeenth intercountry meeting of directors of national and regional polio reference laboratories in the Eastern Mediterranean Region was held in Amman, Jordan on 26–28 January 2015. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Jordan, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisia attended the meeting. Participants also included scientists from the Centers for Disease Control and Prevention (CDC), United States of America; the National Institute for Biological Standards and Control (NIBSC), United Kingdom; the National Institute of Public Health and the Environment (RIVM), Netherlands; the National Institute for Health and Welfare, Finland; and Kenya Medical Research Institute (KEMRI), along with staff of WHO headquarters and the Regional Office for the Eastern Mediterranean.

The WHO global and regional polio laboratory coordinators started the presentations by reviewing the current state of the global polio eradication initiative (GPEI) giving an overview of the performance, activities and challenges faced by WHO network laboratories and describing planned endgame strategies and current priorities for the programme. The performance of the regional poliovirus laboratory network during the period October 2013 to December 2014 was reviewed. Transmission links between wild polioviruses (WPVs) isolated during this period from both acute flaccid paralysis (AFP) surveillance and supplementary surveillance activities were discussed.

The polio eradication programme in the Eastern Mediterranean Region presents specific challenges as problems with security, inaccessibility of children, threats to the lives of polio workers, conflict and political turmoil continue. As a consequence, there are still two endemic countries in the Region that have never eliminated

indigenous WPV transmission and which, in fact, have reported a sharp rise in the number of polio cases during this 2014. Furthermore, polio cases occurred in the Syrian Arab Republic in late 2013 due to the deterioration of public health services, and in 2014 transmission from Syria to Iraq was also reported, resulting in two paralytic cases. However, the large outbreak occurring in Somalia since April 2013 appears to be under control with no cases reported since August 2014. WPV type 3 (WPV3) continues to be absent from AFP cases and environmental samples since it was last reported in Pakistan in April 2012, indicating that circulation of WPV3 might have been successfully interrupted in the Region.

Performance in the Region continues to be high, with laboratories providing high quality information on poliovirus isolation and characterization in a timely manner which is essential for guiding global polio eradication initiative activities. Laboratories in the Region are also actively engaged in supporting endgame activities such as conducting stool surveys from immunodeficient patients, seroprevalence studies or pilot testing of improved laboratory methods. Concerns remain in some aspects of laboratory work, particularly when laboratories are required to handle high workloads unexpectedly as a consequence of a polio outbreak or competing priorities such as influenza pandemics or the recent Ebola outbreak. Contingency plans to respond to these emergencies have not yet been devised in most countries and are urgently needed. Similarly, programmes for biorisk management have not been fully implemented in all laboratories. The importance of laboratory containment as required by WHO guidelines and priorities established in the GPEI endgame strategy plan was also discussed. The 3rd Edition of the Global Action Plan (GAP III) for poliovirus containment has recently been published and describes the different phases that should be

completed according to the progress of the GPEI. As a consequence, new diagnostic approaches might be required and WHO network laboratories will have to respond to increasing demands for rapid and accurate results.

Recommendations were made to address all points discussed above and to sustain and improve the performance in regional laboratories.

2. Summary of discussions

2.1 Status of polio eradication

Although there was a very rapid decline in the number of polio cases and infected countries soon after the GPEI was launched in 1988, achieving complete interruption of poliovirus circulation is proving to be difficult and between 400 and 2000 polio cases have continued to occur since 2000. This has been mainly due to a few remaining countries still endemic for polio failing to immunize children, which results in the occasional importation of WPV in polio-free areas where immunization and surveillance activities are suboptimal. As a consequence, 223, 416 and 359 polio cases occurred globally in 2012, 2013 and 2014, respectively. The rise in the number of cases in 2013 was mainly due to the large outbreak in Somalia, Kenya and Ethiopia which seems to be under control as no cases have been reported from any of these countries since August 2014. There was a sharp rise in the number of polio cases in Pakistan and Afghanistan in 2014 with a total of 334 cases compared to 107 cases in 2013.

During 2013–2014 there was evidence of international transmission of WPV type 1 (WPV1) and on 5 May 2014 the Director-General of WHO declared the international spread of WPV a public health

emergency of international concern (PHEIC), as ten countries had active WPV transmission that could spread to other countries through population movements. During the period January to June 2014, WPV spread internationally in three major epidemiologic zones: in central Asia (from Pakistan to Afghanistan); in the Middle East (Syria to Iraq); and in central Africa (from Cameroon to Equatorial Guinea, and from Equatorial Guinea to Brazil). Furthermore, WPV1 strains related to viruses circulating in Pakistan were found in environmental samples in Israel from February 2013 to April 2014, indicating widespread virus circulation in a population with >95% vaccine coverage with inactivated polio vaccine (IPV) and the absence of known paralytic cases. A number of measures were introduced to reinforce immunization campaigns and enhance surveillance. The situation soon improved in Africa and the Middle East and no polio cases have been reported from these regions since July 2014 and April 2014, respectively. Furthermore, no WPV1 have been isolated from environmental samples in Israel and Palestine since 30 March 2014. However, there has been additional spread in central Asia as outbreaks due to WPV1 continue to occur in Afghanistan and Pakistan. As a consequence, 93% of all WPV cases globally in 2014 were from Afghanistan and Pakistan. Polio cases due to type 2 circulating vaccine-derived polioviruses (cVDPV2s) have been reported in Nigeria, with an increase from 4 in 2013 to 30 cases in 2014, and Pakistan, with a decrease from 48 in 2013 to 23 in 2014. Two cVDPV2 cases were reported from South Sudan in 2014.

2.2 Polio laboratory network performance

Performance of the regional polio laboratory network remains high despite the numerous difficulties and challenges. All laboratories in the Region are fully accredited and maintain certification-standard

performance indicators efficiently supporting GPEI activities. WPVs and VDPVs 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. Also, some laboratories, such as those in Iraq and Syria, are performing under dangerous security situations. During 2014, 94% of isolations of polioviruses and 95% of intratypic differentiation (ITD) results in the regional laboratories were obtained within the required timelines. A total of 37 111 specimens from all surveillance activities were processed. Of those, 30 883 were faecal specimens from AFP cases. Environmental surveillance continues in Egypt and was expanded in Pakistan and Afghanistan. The Region continues to recognize the importance of VDPV isolation and network laboratories in Egypt, Islamic Republic of Iran and Tunisia are participating in projects to assess poliovirus excretion by immunodeficient patients.

The need to devise contingency plans to respond to unforeseen increases in workloads due to a polio outbreak, equipment failure and/or to non-polio emergencies requiring the use of resources from the polio laboratory was discussed. Examples from the WHO regional reference laboratories in Kenya and Egypt were presented. The laboratory in Kenya had to cope with a substantial increase in the workload in a very short period of time during 2013, as a massive outbreak due to WPV1 was detected in Kenya, Somalia and Ethiopia. The outbreak subsequently spread to other areas such as eastern Kenya, elsewhere in Somalia and the Somali region of Ethiopia. This caused a major impact in the laboratory in KEMRI, which handles all AFP samples from the three countries as well as those from South Sudan. The laboratory infrastructure was not able to cope with the testing demand. As a consequence, a backlog of samples for testing accumulated and the laboratory quality indicators dropped,

particularly those related to timelines for poliovirus isolation and characterization. This difficult situation was likely one of the main reasons that led to the cross-contamination of stool samples from South Sudan which were initially identified as positive for WPV1 genetically linked to outbreak strains. This incited major concern as it was thought that the WPV1 outbreak had expanded to a much wider area. However, a thorough investigation including nucleotide sequence comparison between virus isolates obtained in the same laboratory and scrutiny of laboratory records clearly identified the virus isolates from South Sudan as cross-contamination from samples from Somalia that had been handled in the same laboratory area at the same time.

The laboratory in Egypt has been an excellent performer for a number of years and has shown a dramatic increase in workload in recent years, as apart from acting as a national polio laboratory for Egypt it also acts as national polio laboratory for Lebanon and Yemen and ITD laboratory for Syria, Iraq and Sudan. The number of samples for testing has increased dramatically in recent years due to more effective AFP surveillance in Egypt leading to an increase in the rate of non-polio AFP cases identified (from 1.0 to 2.9 cases per 100 000 children under 15 years of age), the collection of more samples from contacts of AFP cases, the large number of samples received from Syria and Iraq due to the 2013–2014 outbreak and the involvement of the laboratory in extensive environmental surveillance throughout Egypt as well as in testing stool samples from immunodeficient individuals as part of a regional study. In order to minimize the impact of increasing workloads, the laboratory established separate areas of work for handling samples from different countries such as those from countries infected with WPV or those from countries with long-standing polio-free status. Assigning different activities to different

members of staff has also been found helpful as it is establishing clear lines of responsibility, assigning back-up staff to the different roles and training new people to cope with increases in workloads.

2.3 Virus surveillance

The WHO regional reference laboratory in Pakistan continued to maintain a high level performance during 2014. Laboratory quality indicators such as cell culture results reported within 14 days or ITD results reported within 7 days were well above the minimum requirements from both Pakistan and Afghanistan. Stool samples from 10 426 AFP cases and 1433 contacts were received from Pakistan during 2014. A total of 306 WPV1 and 23 cVDPV2 cases were identified compared to 93 WPV1 and 48 cVDPV2 cases found in 2013. A total of 28 WPV1 cases were confirmed from stools of 4751 AFP cases and 1195 contacts from Afghanistan. The non-polio enterovirus (NPEV) isolation rate was 18% and 17% for Pakistan and Afghanistan, respectively. Three hundred and fifteen environment samples from 36 different sites in Pakistan were also analysed. Out of these, 130 samples were shown to contain WPV1 strains and 9 were positive for cVDPV2 isolates. Environment surveillance in Afghanistan was also initiated at the end of 2013 from three different collection sites, which were then increased to 11 sites in 2014. Seventy-five samples were received from these sites during 2014. Out of these, 16 samples were positive for WPV1.

Although the laboratory has experienced delays in receiving reagents due to blockades at Customs, the laboratory remained active in their contribution to the standardization of various testing techniques including poliovirus diagnostic ITD and VDPV rRT-PCR with CDC kits for environmental/sewage samples, poliovirus diagnostic ITD

rRT-PCR with Quanta Tough Mix kit (Version 2), poliovirus diagnostic ITD rRT-PCR with Quanta Tough Mix kit (Version 4) and VDPV VP1 rRT-PCR with Quanta Tough Mix kit.

One of the major concerns in the Region in 2013 was the detection of WPV1 circulation in Deir Al Zour, Syria. A sudden surge of AFP cases was detected in Deir Al Zour in September–October 2013, all associated with WPV1 isolates related to WPV1 genetic cluster R3 originally found in Pakistan. The virus then expanded to other locations of the country. The total number of confirmed polio cases from this outbreak is 36, with 35 cases in 2013 and 1 case in 2014, with onset 21 January 2014. All major AFP surveillance indicators such as the number of AFP cases detected and the percentage of adequate stool samples collected from AFP cases have declined significantly in the past few years, from 194 in 2010 to 109 in 2012 and from 84.4% in 2012 to 72.7% in 2013, respectively. The last case due to indigenous WPV1 was detected in Syria in 1995. WPV1 was imported from Syria to Iraq resulting in two WPV1 cases, the last one reported from Baghdad with onset on 7 April 2014.

As noted previously, a massive outbreak due to WPV1 was detected in Kenya, Somalia and Ethiopia in 2013. CDC confirmed the isolates as WPV1 genetically related to viruses first seen in Nigeria. The outbreak subsequently spread to other areas such as eastern Kenya, elsewhere in Somalia and the Somali region of Ethiopia. The outbreak appears to be over as only five WPV1 cases were found in Somalia in 2014, 1 in Ethiopia and none in Kenya. The onset of the most recent WPV1 from this outbreak was 11 August 2014 found in Somalia.

2.4 Molecular epidemiology

All WPV1 isolates from Pakistan and Afghanistan belong to the SOAS genotype. More than 88% of all cases in Pakistan and Afghanistan were from security bound areas where there is a vaccination barrier since 2012. A significant number of the cases (85%) occurred in children with less than 2 years of age. Despite the rise in the number of confirmed polio cases, the genetic heterogeneity among isolates from Pakistan and Afghanistan appears to have decreased slightly in 2014 as viruses from 11 and 12 genetic clusters were detected in Pakistan and Afghanistan, respectively, compared to 14 genetic clusters in 2013. Population migration is still regarded as the major source for the transmission of WPVs between different areas in both countries. There were three major outbreak areas in Pakistan: Federally Administered Tribal Areas (FATA), Karachi and Quetta-Killabduallah. WPV1 circulation was re-established in Quetta following importation of WPV1 from Karachi. Poliovirus transmission was also detected once again in Hyderabad, Sukkur and Jacobabad after importation of WPV1 from Karachi. All viruses isolated in Punjab were imported cases either from FATA or Quetta-Killabduallah. Viruses isolated from environment samples in Lahore indicated that local transmission occurred in the area during 2014. One virus isolate found in an environmental sample from Hyderabad in May 2014 showed 2.1% VP1 sequence divergence from its closest match (a 2012 environmental isolate from Peshawar). Another environmental sample from Karachi in July 2014 showed 2.1% VP1 sequence divergence from the 2014 Hyderabad environmental isolate suggesting active silent transmission of viruses from this genetic cluster (R4B7).

Most of the viruses found in Afghanistan were imported from Pakistan. However, two orphan viruses belonging to genetic cluster R4B1 were isolated from AFP cases in Uruzgan and Helmand, in May and October 2014, respectively, and were genetically linked to 2012 Afghanistan viruses indicating the silent circulation of virus in southern Afghanistan. Another orphan virus from genetic cluster R2A was found in an environmental sample from Quetta (Pakistan) collected in August 2014, which showed 3.6% VP1 sequence divergence from its closest known matches, a 2012 environmental isolate from Peshawar and a 2012 AFP isolate from Khyber. This orphan virus was very closely related to a virus found a month later in an AFP case from Panjwai, Kandahar (Afghanistan). Lastly, an orphan virus of genetic cluster R4B1, with 1.55% VP1 sequence divergence from a 2013 isolate from Khyber (Pakistan) was also found in Laghman (Afghanistan). Viruses from genetic cluster R4B5 were the most prevalent in both countries in 2013 and 2014. Most environmental isolations correlated with AFP case distribution. Viruses from 9 and 4 genetic clusters were found in environmental samples from Pakistan and Afghanistan, respectively. All WPV1 isolates from 2014 in Somalia were related with the 2013 viruses, from WEAFF-B1 cluster N5A originally found in Nigeria.

The first emergence of a cVDPV outbreak in Afghanistan and Pakistan was recorded in 2009, starting from a cVDPV2 isolate from an AFP case in Nade Ali, Helmand (Afghanistan), which contained 9 VP1 nucleotide differences from the Sabin 2 vaccine strain. This outbreak occurred between 2009 and 2013. Up until the end of 2014, eight type cVDPV emergences have been identified in these two countries all linked to serotype 2 viruses. Out them, seven emerged in Pakistan and one in southern Afghanistan. The first cVDPV2 emergence in Pakistan occurred in Killa Abdullah and it is the largest

emergence in both countries still occurring in 2014. Viruses from this emergence were also imported to Karachi and Waziristan North in Pakistan and to southern Afghanistan. Waziristan North also account for four different type cVDPV2 emergences (two of them active in 2014) whereas one emergence occurred in the Gadap community of Karachi (also active in 2014).

Immunodeficient VDPV (iVDPV) isolates from AFP samples of 2 immunodeficient patients were identified in the Islamic Republic of Iran in 2014. Samples corresponded to two males one with X-Linked Agammaglobulinemia and another with still unknown syndrome. Four type 1 (1.8% VP1 sequence divergence from Sabin 1) and four type 2 (0.6% VP1 sequence divergence from Sabin 2) isolates were obtained from these cases, respectively. Subsequent samples from both cases were negative for poliovirus. Six samples from another male suffering from severe combined immunodeficiency (SCID) were positive for iVDPV1 containing between 2.44% and 3.30% VP1 sequence divergence from Sabin 1. Three subsequent samples tested were found to be negative for poliovirus. Three ambiguous VDPV (aVDPV) isolates were found in environmental samples in Egypt two aVDPV1 from Al Wasla (Giza) and Banha (Qaliobia) and one aVDPV2 from Alharam Giza).

2.5 Environmental surveillance

Environmental surveillance is used to supplement AFP surveillance, sampling populations rather than individuals. It represents a powerful tool to detect wild or VDPV circulation and to guide further surveillance activities and immunization campaigns. Environmental surveillance has been used to demonstrate the polio-free status of IPV-using countries and more recently, to detect the reintroduction of

WPV and VDPVs into polio-free areas even in the absence of AFP cases, as shown recently in Israel and Palestine. The current sewage concentration method recommended by the WHO global poliovirus laboratory network is the two-phase separation based on delicate physicochemical interactions between two carbohydrate polymers (dextran T40 and polyethyleneglycol 6000) in solutions and the virus particles. There are other methods described in the literature and laboratories of the global poliovirus laboratory network are actively testing different techniques with a view to complete WHO guidelines that will include detailed standard methods and accreditation procedures to ensure quality assurance.

Afghanistan, Egypt and Pakistan have implemented environmental surveillance to detect polioviruses in sewage water in strategic locations. The regional reference laboratory in Egypt was a pioneer in implementing this technique. It started with 2 provinces, 5 sites and 5 samples per month and now in 2014, environmental surveillance for poliovirus is conducted in 22 provinces, 38 sites and 40–42 samples per month. Samples are collected from border provinces to detect importation of VDPV or WPV. Extra samples are collected in case of detection of imported VDPV or WPV and new sites can be added according to programme needs. Environmental surveillance played an important role in documenting the interruption of endemic WPV circulation in Egypt. In December 2012, WPV1 was isolated from two sites in Cairo (Alsalam and Alhaggana). The virus was genetically linked to WPV1 circulating in Sindh, Pakistan, during 2012 and was later found in environmental samples in Israel and Palestine and AFP cases in Syria and Iraq. Twenty-seven aVDPV isolates have been found in environmental samples from Egypt between 2004 and 2014, three of them in 2014.

Since 2009, environmental surveillance to detect polioviruses in sewage water samples is conducted in strategic locations in Pakistan. Currently, 36 active environmental surveillance sites are operating in 12 districts across 4 provinces. There are plans to expand to new 5 sites for a total of 41 sites before the end of 2015. A total of 931 samples were tested between 2012 and 2014, 59% of them were shown to contain Sabin viruses, 31% WPV isolates, 9% NPEV and 1% VDPVs. Environmental surveillance was initiated in Afghanistan in 2013 in two cities of Kandahar (Khandak and Rarobat). Currently, there are 11 active sites in 6 districts/towns. There are 10 new proposed sites for a total of 21 by the end of 2015. Between 2013 and 2014, 15 WPV, 63 Sabin and 15 NPEV were isolated from environmental samples in Afghanistan. WPV and VDPV isolates from most genetic clusters found in AFP samples are regularly identified in environmental samples from Afghanistan and Pakistan.

2.6 Laboratory quality assurance

Annual proficiency testing and assessment of laboratories continue to be critical for the quality assurance of the performance in polio laboratories. Four different proficiency testing panels are in use for evaluating: a) accuracy of virus isolation; b) ITD by real-time PCR (rRT-PCR), c) rRT-PCR for VDPV screening and d) sequencing poliovirus isolates. The proficiency testing programme is coordinated by WHO in collaboration with the global specialized laboratories in the United States and the Netherlands. A standardized test to measure the sensitivity of the 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 all relevant laboratory techniques. New accreditation checklists have been introduced and tested with good experience which has allowed

streamlining the accreditation process. However, there is a risk of decline in performance due to changes in public health priorities and competing priorities. That is why performing proficiency testing regularly remains to be critical.

The distribution of the annual proficiency test for isolation of polio- and enteroviruses to all the 148 member laboratories of the global polio laboratory network was a challenge in 2014: increasingly tight restriction on shipment of infectious materials, in combination with transfer bans in several countries because of the Ebola crisis. The distribution of proficiency tests in the Region was no exception. Some shipments were sent back to RIVM by the shipping company. Other shipments took up to 5 weeks before arriving at final destination. In the end, all 12 regional laboratories were able to perform the test, some however only in 2015. The proficiency test scores for the 12 laboratories were as follows: nine laboratories reached a perfect 100% score, as all reported results were correct. One laboratory reached a 90% passing score: most likely two poliovirus-negative samples were exchanged. The Regional Laboratory Coordinator will check the correct implementation of corrective measures taken to overcome the weakness during his next onsite visit. Two laboratories missed the poliovirus in sample 10 for different reasons. One laboratory reported the correct result upon reanalysis of the sample; a new proficiency test was shipped to this laboratory from VACSERA via the Regional Office. Results are yet to be reported. For the second laboratory it cannot be excluded that missing the polio component in sample 10 was due to inactivation of the virus during the rather complicated and long lasting transport of the proficiency test under suboptimal conditions. Further analysis with longer incubation times than recommended in the new algorithm showed growth on L20B in at least one tube. This finding indicates a much lower live virus

concentration in the sample than at the time of preparation. The performance of the regional laboratories in the annual proficiency testing for isolation of polio and enteroviruses over the last 24 years has been excellent. Despite having to face increasing challenges (e.g. increased numbers of samples to be tested 5 to >10, increased passing score from 80 to > 90%, implementation of a new algorithm with shorter reporting times), higher mean scores were achieved by regional laboratories in recent years. These developments document the high and up-to-date performance quality of the regional laboratories, which are able to face all challenges that come on the way to successful eradication of poliovirus circulation in the Eastern Mediterranean Region and the world.

There were some challenges in the proficiency test panel for rRT-PCR ITD as an enterovirus-only sample gave a Sero PV2 positive result in some laboratories. This was found to be due to poor RNA and variations in machine sensitivity. This sample was discarded for further analysis. Some laboratories also struggled with a sample containing a mixture of P1NSL+P3NSL. Following troubleshooting procedures recommended by CDC should help solving the few minor problems encountered in some laboratories such as adjusting baseline settings and y-axis to visualize the data curves clearly. regional laboratories are highly proficient in polio PCR assays, turnaround time is excellent for all laboratories and proficiency test results correlate with routine results.

Interpretation remains the biggest issue throughout the global poliovirus laboratory network. Report letters with proficiency test results for all laboratories should be sent soon.

The two regional laboratories performing sequencing of poliovirus isolates, in Pakistan and Tunisia, passed the 2014 poliovirus diagnostic sequencing proficiency test panels, with both laboratories reporting sequences identical to the reference sequences, standard timelines met and excellent results and interpretation. There were only very minor issues found which did not affect the results.

On the negative side, Syria and Iraq continued having problems in achieving <80% the referral of >80% poliovirus isolates/L20B positive to the regional reference laboratory within 7 days.

2.7 Development and evaluation of new diagnostic methods and reagents

Regional reference laboratories and particularly global specialized laboratories continue to contribute to the validation, implementation and improvement of methods used by the global poliovirus laboratory network. This is particularly relevant in the context of planned changes for the post-eradication and post-OPV eras which will include a switch from trivalent OPV to bivalent OPV, the introduction of global IPV use and, eventually, the total interruption of OPV immunization.

Scientists from CDC are continuously updating protocols for ITD rRT-PCR as there is a constant demand for better sensitivity and specificity to quickly identify viruses for sequencing and to allow direct screening during outbreaks. There are evolving diagnostic questions due to the eradication of WPV2, the need for detecting VDPVs and the importance of sorting out virus mixtures as we cannot afford to miss a WPV in a homotypic mixture. In this regard, establishing improved ITD rRT-PCR methods will be very useful as

this technique will be routinely used for the characterization of poliovirus isolates from environmental samples and eventually for the direct detection of poliovirus isolates from stool samples. Regional network laboratories have contributed to the pilot-testing and validation of rRT-PCR ITD version 4.0 which includes reactions for EV/Sabin-multiplex, Pan-PV, WPV1-multiplex, WPV3-WEAF-B and WPV3-SOAS. New kits and buffers for PCR reactions have been also tested to increase the sensitivity for poliovirus detection. Optimal testing algorithms for the isolation and characterization of poliovirus from environmental samples are also being devised.

3. Recommendations

1. Laboratory directors should prepare, in coordination with the regional coordinator and national authorities, a contingency plan to respond to unforeseen increases in workloads due to a polio outbreak, equipment failure and/or to non-polio emergencies requiring the use of resources from the polio laboratory. To achieve this:
 - a. The contingency plan should include all aspects that contribute to successful laboratory performance such as human resources, physical facilities, supplies, testing strategy, data management, communication, coordination, funding and biorisk measures. The regional coordinator should send a template to laboratories to help with process. Laboratory directors should send a draft plan to the Regional Office before 1 June 2015.
 - b. The Regional Office should consider redistributing part of the workload from laboratories with high demands to laboratories that have experienced very low workloads for years. This will

- also ensure that these laboratories achieve levels of work required to maintain adequate competency.
2. Maintaining high levels of performance by laboratories of the WHO regional polio laboratory network continues to be a priority in a context of the current political situation, competing public health priorities and budget constraints. To achieve this:
 - a. The Regional Office should continue to work closely with governments and donors to ensure adequate logistics, human and financial resources are available for the regional laboratory network.
 - b. Regional and national reference laboratories should maintain frequent communication between themselves and the Regional Coordinator to help monitoring performance, facilitate shipment of poliovirus isolates for ITD characterization and ensure correct reporting of results and follow-up actions. The laboratory should send a quarterly report to collaborating laboratories and the regional coordinator.
 - c. The interpretation of molecular epidemiological data generated by laboratories should be coordinated by regional/global laboratory coordinators as soon as available and scientists from the global specialized laboratories to facilitate that accurate chronological and geographical links are established between poliovirus isolates from different areas, country or regions as this provides essential information to guide GPEI activities.
 3. Laboratories are encouraged to participate in research and development projects to support the GPEI which include iVDPV surveillance, vaccine immunogenicity and seroprevalence studies. The regional coordinator and laboratory directors should work together to identify expertise available in network laboratories that

can be used in other global health programmes as part of the global poliovirus laboratory network legacy workstream. This should be done in consultation with programme partners, donors and national ministries of health.

4. Environmental surveillance activities provide essential information to the programme, particularly in the context of the endgame for poliovirus eradication and GAP III recommendations, as this method is known to be very sensitive in detecting poliovirus circulation even in the absence of known paralytic cases. There is a need to consolidate and expand these activities in the Region where appropriate. To achieve this:
 - a. The Regional Office should provide laboratories with revised guidelines for environmental surveillance defined by WHO headquarters. The Regional Office should continue to work closely with governments and donors to assist in implementation and expansion of environmental surveillance without compromising the timely testing of AFP samples.
 - b. The Regional Office and regional polio network laboratories should work together to cover needs for testing environmental samples, including training, when the implementation or expansion of this technique is required by the country.
5. Training for biorisk management and promoting biosafety practices in all laboratories should be a high priority for the WHO Regional Office. To achieve this:
 - a. The Regional Office should ensure appropriate training activities are conducted to ensure successful establishment of a biorisk management system.
 - b. Laboratory directors should implement a biorisk management system in their laboratories, in coordination with local and national authorities and collaboration with WHO headquarters and the Regional Office.

- c. The biorisk management system should include a clear definition of roles and responsibilities related to biorisk practices.
6. Laboratory containment activities should continue as required by WHO guidelines and priorities established by the GAP III document. To achieve this:
 - a. Regional laboratories should serve as models and therefore destroy WPV whenever possible. A procedure for destroying polioviruses will be included in the polio laboratory manual.
 - b. Attention must be paid to the implications of OPV2 withdrawal for containment. Rapid and comprehensive detection of VDPV2 is a prerequisite for the switch from tOPV to bOPV.
7. Maintaining high levels of laboratory quality assurance according to the WHO accreditation process is essential. To achieve this:
 - a. Laboratory directors should ensure that all staff complete appropriate training for all required laboratory techniques. Hardcopies of training records and CVs for all staff members should be maintained. Records should include demonstration of competence in laboratory techniques, academic records, training in biosafety, etc.
8. Regional network laboratories should implement the improved rRT-PCR ITD 4.0 method following recommendations by the polio small working group (SWG) and WHO headquarters. To achieve this:
 - a. The WHO Regional Office, in coordination with WHO headquarters, should facilitate the timely provision of required reagents to perform this technique.
 - b. Laboratories should follow instructions and standard operation procedures to implement and validate the new technique as instructed by the SWG.

9. Ensuring continuous flow of high quality data is essential to assess and follow up laboratory performance. To achieve this:
 - a. The Regional Office needs to complete the upgrading of the LABIFA software and ensure it is installed and functional in all laboratories.
 - b. Regional network laboratories should make use of the different LABIFA modules to ensure they provide high quality data to the programme and help adequate follow-up of laboratory performance and timeliness.
 - c. Laboratories conducting environmental surveillance should use the new module in LABIFA to standardize data compilation in the Region and improve quality.



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