

Summary report on the

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

Virtual meeting
10–11 November 2021



REGIONAL OFFICE FOR THE

World Health
Organization

Eastern Mediterranean

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1. Introduction

The twenty-second intercountry meeting for directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held virtually on 10 and 11 November 2021. The meeting was attended by the directors of national poliovirus laboratories from 12 countries: Egypt, Iran (Islamic Republic of), Iraq, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Syrian Arab Republic, Sudan and Tunisia. Participants also included members of national certification committees (NCC) and the Regional Certification Commission of the Eastern Mediterranean Region for polio eradication (RCC), national polio containment coordinators and technical experts from the US Centers for Disease Control and Prevention (CDC), UK National Institute for Biological Standards and Control, (NIBSC), Netherlands National Institute of Public Health and the Environment (RIVM), Kenya Medical Research Institute (KEMRI), Bill and Melinda Gates Foundation (BMGF) and Yemen's Central Public Health Laboratory, along with staff from WHO headquarters and the Regional Office for the Eastern Mediterranean and an independent consultant from Finland.

The objectives of the meeting were to:

- review the poliovirus regional laboratory network's performance;
- brief on the Global Polio Eradication Initiative's (GPEI's) polio eradication strategy 2022–2026;
- discuss the roll out of new testing methods and techniques in the Region, with a focus on the novel oral polio vaccine (nOPV2) and other innovations;
- discuss the role of polio laboratories in the polio endgame strategy, Global Action Plan III (GAPIII), Phase 1 activities and environmental surveillance (ES); and
- develop recommendations for further improvement in laboratory performance.

Dr Hamid Jafari, Polio Director for the WHO Regional Office for the Eastern Mediterranean, welcomed participants and commended the contribution of the Eastern Mediterranean Regional Polio Laboratory Network (EPLN) in support of surveillance activities and early detection of polioviruses for a timely response in the field. He also praised the excellent collaboration among EPLN and laboratories in WHO's European and African regions for ongoing work and dealing with emergencies. He applauded the high level of commitment and progress made towards the eradication of poliomyelitis in the Region during 2021 and encouraged the EPLN to continue to support acute flaccid paralysis (AFP) surveillance for polio eradication activities.

2. Summary of discussions

The meeting reviewed the recommendations from the previous intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region and expressed satisfaction with their implementation.

Global and regional progress in polio eradication

The only two wild poliovirus 1 (WPV1) endemic countries, Afghanistan and Pakistan, which have never eliminated indigenous WPV1 transmission, are in the Eastern Mediterranean Region. Furthermore, the polio eradication programme in the Region faces specific challenges such as problems with security, conflict and political turmoil, the inaccessibility of children and threats to the lives of polio workers. Recommendations were made to sustain and improve the performance of regional laboratories.

The EPLN is working optimally and maintaining the certification of standard performance indicators for polio endemic, outbreak and polio-free countries. Compared to 2020, in 2021 there was a significant

decline in detection of cases, with WPV1 transmission at historically low levels. Furthermore, a significant decline in WPV1 genetic diversity was seen and fewer clusters were identified. However, continued detection of orphan viruses might be indicative of surveillance gaps, particularly in very high-risk districts. Circulating vaccine-derived poliovirus type 2 (cVDPV2) continued to emerge and circulate, with the isolation of 13 emergences globally including six in the Region during the last six months. The increased number of cVDPV2 outbreaks has led to a corresponding increase in the workload of Global Polio Laboratory Network (GPLN).

The global polio eradication strategy 2022–2026 highlights the need to focus on addressing the remaining gaps in surveillance and immunization, timely detection and response to WPV1 and vaccine-derived poliovirus (VDPV) outbreaks and continued alignment with the Global Surveillance Action Plan (GSAP) to meet programmatic needs through new tools to achieve a polio-free world. The priority set by the polio laboratory network is fast tracking the validation and stepwise implementation of the new direct detection (DD) methodology for AFP specimens by the fourth quarter of 2022. The EPLN is part of the pilot testing of the DD method.

It was noted that the EPLN had provided significant logistical, technical and human resources support to the COVID-19 pandemic response. This was highly commended and appreciated. Network laboratories had worked under tremendous pressure during the acute onset of the pandemic and had continued to provide support to ensure adequate attention to the backlog of sample testing and shortage of supplies. Despite these constraints they continued to provide high quality results to the polio eradication programme.

Laboratory network performance in endemic and outbreak countries

The quality and performance indicators for EPLN laboratories have been maintained at high certification standards despite the numerous difficulties and challenges including war, conflict and insecurity. The ongoing COVID-19 pandemic has adversely impacted the work of the laboratory network, including disruption in sample shipment, repurposing of polio laboratories for COVID-19 activities leading to delayed detection of polio outbreaks in the Region, human resource constraints and critical shortages of consumables and reagents. Despite these challenges, the network laboratories have continued to support polio eradication activities. All network laboratories were accredited through desk review due to travel restrictions this year. All laboratories, except one, passed the WHO proficiency testing panel of unknown viruses for primary virus culture. Six out of ten intratypic differentiation (ITD) laboratories participated in ITD of the VDPV proficiency testing (PT) panel, and all secured a passing score.

Afghanistan and Pakistan are the only endemic countries in the world. In both countries, there was persistent WPV1 circulation of the SOAS genotype in core reservoir districts. Overall, WPV1 transmission in the Region is at an historic low level and is confined to Pakistan and Afghanistan, with a significant decline in genetic diversity. While there is also a decline in cVDPV2 detection in Afghanistan and Pakistan, the risk of international spread of VDPV2 from the countries remains high. From October 2020 to February 2021, five different events of cVDPV2 importation of PAK-GB-1 emergence occurred in the bordering provinces of the Islamic Republic of Iran.

The cVDPV2 outbreak reported in Sudan was due to the CHA-NDJ-1 emergence imported from Chad, resulting in 58 cases in 2020. No case was reported in 2021. The cVDPV2 CHA-NDJ-1 emergence circulating

in Sudan spread to Egypt in January 2021; it was detected in AFP cases and in environmental samples. A total of 11 cVDPV2 isolates were detected from sewage water samples taken from Alexandria, Aswan, Beni Suif, Kafr AlSheikh, Giza and Qena, while no isolation was detected through AFP surveillance. After implementation of monovalent oral poliovirus vaccine 2 (mOPV2) campaigns, a total of 67 VDPV2 isolates were collected from sewage water samples in 26 provinces between April and October 2021. The VDPV2 isolates range from a 6 to 13 nucleotide difference in VP1 from the Sabin 2 prototype.

In the Eastern Mediterranean Region, as of November 2021, a total of three WPV1 cases were reported (one from Pakistan and two from Afghanistan), alongside 64 cVDPV2 cases (44 from Afghanistan, nine from Pakistan, 10 from Egypt and one from Somalia), nine aVDPV2 cases (one from Afghanistan and eight from Pakistan) and three cVDPV1 (Yemen) cases, detected as AFP. In the same period, the ES system detected 68 WPV1 isolates (67 from Pakistan and 1 from Afghanistan), 135 cVDPV2 isolates (82 from Pakistan, 49 from Afghanistan and four from Islamic Republic of Iran) and 142 VDPV2/aVDPV2 isolates (67 from Pakistan, 65 from Egypt, eight from Afghanistan and two from Islamic Republic of Iran).

The regional reference laboratory (RRL) in Pakistan serves as a national and regional laboratory for both Afghanistan and Pakistan. It has tested more than 26 000 stool samples from AFP cases and 1265 environment samples from both countries so far during 2021. Pakistan currently has an extensive ES network of 65 sites and Afghanistan has 29 sites, covering mostly the high-risk and vulnerable districts across the country. ES has contributed significantly by detecting the virus in its initial stages, allowing the programme to respond quickly to new circulations and outbreaks.

The laboratory continued to detect poliovirus with speed and accuracy despite the rise in workload. The poliovirus serology laboratory is supporting the polio eradication programme by testing sera for poliovirus antibodies. In addition, the staff at the RRL in Pakistan is well trained in other areas of viral diagnostics. They have supported COVID-19 testing, provided training and facilitated the establishment of COVID-19 diagnostic capacity in other provinces of Pakistan with the support of National Institute of Health in Islamabad.

VACSERA, the national poliovirus laboratory (NPL) in Egypt, has maintained an excellent performance over many years. There was an increase in workload due to the VDPV2 outbreak. The laboratory has tested 3000 stool samples received through AFP surveillance and the primary immune deficiency disorder (PID) project. VACSERA is an RRL providing virus isolation, ITD and nucleotide sequencing facility to Iraq, Lebanon, Syrian Arab Republic, Sudan and Yemen.

In Egypt, AFP surveillance is supplemented with ES, which is providing high quality information and adding value to poliovirus surveillance sensitivity. VACSERA tested more than 500 environment samples in 2021 from 47 well-established collection sites covering 27 governorates.

The KEMRI poliovirus laboratory in Kenya supports both AFP and ES sample testing for Djibouti, Somalia and Yemen. Historically, the performance of the laboratory has been good and all performance indicators on sample numbers, timeliness and accuracy, proficiency testing and reviews have been consistently good. The laboratory performance indicators have maintained a high standard, except for the timelines of reporting, due to renovation and delays in supplies due to the impact of COVID-19 and equipment breakdown.

Environment surveillance for polioviruses

Poliovirus ES has been used to supplement AFP surveillance. It has proved its added value in early detection of WPV/VDPV and guided both surveillance and immunization responses. One of the major objectives of the global polio surveillance action plan (GPSAP) 2022–2023 is the optimization of the ES network and maintaining and strengthening the capacity and capability of the GPLN to contribute to the timely detection of polioviruses. Currently, ES is performed using global site-specific indicators that include: > 50 % of samples isolated enteroviruses (EV); 80% of samples arrive in the laboratory in good condition; 80% of samples are collected at recommended hour of day; and 80% of samples arrive in the laboratory within three days for in-country, or seven days for international shipment, of collection. Additionally, there is a need for implementation and expanded use of the Web-IFA electronic database tool to improve data quality and accuracy.

ES has been implemented in Afghanistan, Djibouti, Egypt, Islamic Republic of Iran, Jordan, Kuwait, Lebanon, Pakistan, Syrian Arab Republic, Sudan and Yemen, to detect polioviruses in sewage water in strategic locations. In response to cVDPV2 outbreaks, multiple ad hoc sewage water collection sites have been opened in both Afghanistan and Pakistan. Additionally, in many high-risk areas, the frequency of sample collection has been changed from monthly to fortnightly.

A study using the bag mediated filtration system (BMFS) was conducted as a collaboration between PATH and the National Institute of Health RRL in Pakistan. During 2021, 122 sewage samples have been collected from 12 sites including Bannu, Rawalpindi, Peshawar, Faisalabad, Lahore, DG Khan, Karachi Landhi, Jacobabad, Hyderabad, Quetta and Rahim Yar Khan. As of 8 November 2021, 15 samples taken from Peshawar, Multan, Lahore, Rawalpindi, Faisalabad, Hyderabad

and Bannu were positive for WPV1, three with both WPV1 and cVDPV2 isolations taken from Karachi and Quetta and seven for aVDPV2 taken from Rawalpindi, Lahore, Multan and Jacobabad.

New opportunities: progress on direct detection (DD) of PV

The current gold standard method of poliovirus detection involves viral culturing in a combination of two cell lines, intratypic differentiation (ITD) by reverse transcription-polymerase chain reaction (RT-PCR) to determine serotype by distinguishing Sabin from non-Sabin polioviruses and subsequent sequencing by traditional methods such as Sanger. However, while this method is sensitive for poliovirus detection, it is lengthy and may lead to delays between sample collection and final sequencing result, compromising the speed and effectiveness of any vaccination response. In addition, the WHO Global Action Plan III (GAP III) aims to minimize the handling and isolation of live PVs, particularly PV2. Hence, GPLN laboratories require switching to a culture-independent diagnostic method (DD) that is safe, fast, robust and at least of equivalent sensitivity to the cell-culture procedure.

The CDC, Atlanta, has developed a sensitive direct detection method for detection of polioviruses from stool samples, and initial data supports that this method is non-inferior to the current culture method. The method is amenable for implementation in a wide variety of polio laboratories and is currently under validation process. The direct detection algorithm involves real time RT-PCR after RNA extraction directly from stool suspension using the same molecular ITD method currently used for identifying PVs from cell culture samples. Efficient RNA extraction methods for PV have been identified and include a manual method using the Zymo Research quick RNA extraction kit, and the automated extraction method using the Roche nucleic acid extraction kit with modified Kingfisher Duo. A Q β virus standard is used as an internal

control for validation of the RNA extraction process. Automation streamlines laboratory work, reduces contamination and is cost-efficient. Proposed sequencing modules for the direct detection demonstration project include sequencing both direct and nested VP1 PCR products using the classic Sanger method and a novel next generation sequencing (NGS) nanopore procedure developed by Shaw, et al.

NGS of poliovirus genomes can provide multiplexing ability by sequencing homotypic or heterotypic PV mixtures. NGS can be used at different stages, including detection from stool, viral culture and intratypic differentiation stage of the GPLN's poliovirus algorithm. The method for direct detection of poliovirus developed by Shaw et al. involves a nested PCR and nanopore sequencing protocol that allows rapid (< 3 days) and sensitive direct detection and sequencing of polioviruses in stool and environmental samples providing a faster and safer alternative to cell culture. Barcoded primers and a real-time analysis platform were developed that generate accurate VP1 consensus sequences from multiplexed samples. Additional testing, validation and training of the nanopore direct detection method is underway in GPLN laboratories and further development of bioinformatic software for identifying PV mixtures in ES samples is ongoing.

The virology laboratory at the Central Public Health Laboratory in Sanaa, Yemen, has been identified as laboratory from the EPLN where the DD method of testing could be established. Given the complexities and challenges to shipping samples to other GPLN laboratories, establishment of this laboratory will decrease the turnover time of PV detection, which is critical for the programme to enable fast outbreak response and quickly stop transmission. Staff from the Central Public Health Laboratory will be trained at the RRL in Pakistan. Furthermore, there is a plan for the establishment of a DD laboratory in Afghanistan (from the EPLN) to support the programme in early PV detection and

timely action to control virus circulation. This facility will strengthen the health surveillance system of the country and may be used for the detection of other emerging pathogens during an epidemic or pandemic.

Novel OPV2 (nOPV2) genetic characterization background and laboratory perspectives and roll out plan

The limitations of existing vaccines and current eradication challenges, including VDPV emergences, warranted the development of more a genetically stable OPV strain, most urgently for OPV2. A novel OPV2 has been developed that incorporates mutations to increase genetic stability. The new vaccine has been shown to have increased attenuation with respect to Sabin OPV2, to be antigenically indistinguishable from Sabin OPV2, to induce neutralizing antibodies as effectively as Sabin OPV2, and, unlike Sabin OPV2, has been shown to be genetically stable and to maintain an attenuation phenotype. nOPV2 was the first vaccine granted emergency use listing (EUL) by WHO in November 2020 and has already been used in several countries to respond to cVDPV2 outbreaks.

Enhanced monitoring of the evolution of nOPV2 in humans is required while nOPV2 is used under EUL to assess safety surveillance, performance, quality complaints and other relevant factors impacting the validity of the listing. Essential criteria for the initial use of nOPV2 requires the availability in GPLN laboratories of an algorithm and testing capacity for the detection of nOPV2 in stool and sewage samples. An ITD qRT-PCR kit version 6.0, including duplex assay for nOPV2 detection, has been developed by CDC. All nOPV2 positive samples need to be further sequenced through the whole genome by NGS to identify nOPV2 genetic markers and analyse the genetic stability of the vaccine.

Eight countries from the Eastern Mediterranean Region, including Afghanistan, Djibouti, Egypt, Iran (Islamic Republic of), Iraq, Pakistan, Somalia and Sudan, have expressed their interest in using nOPV2 after appropriate training and preparation of the requirements for the nOPV2 use under EUL in case of an cVDPV2 outbreak. Seven countries (Afghanistan, Djibouti, Egypt, Islamic Republic of Iran, Pakistan, Somalia and Sudan) have completed all the required documents and have been verified by the global Readiness Verification Team (RVT). Iraq has submitted all the required documents and is in the process of verification. Training on nOPV2 detection algorithm and reporting was provided to all laboratories in the Region with the support of CDC. The laboratories are equipped with sufficient stock of reagents, kits and consumables needed for detection of nOPV2 and are prepared to ship samples to CDC, US, or NIBSC, UK, for whole genome sequencing.

Implementation of the Global Action Plan III (GAPIII) in the East Mediterranean Region

The polio endgame strategy 2019–2023 and polio eradication strategy 2022–2026 focus on: poliovirus detection and interruption, health system and immunization strengthening, containment of PV materials and certification. In accordance with the GAPIII for the containment of polioviruses, the destruction of WPV2 and VDPV2 material has been completed in all facilities of the Region. Destruction of PV2 material identified during the ongoing cVDPV2 outbreak in the Region is in progress and laboratories handling the PV2 positive material have confirmed its destruction. The Region's electronic web-based poliovirus containment database management system is functional and countries are encouraged to enter laboratory survey and inventories of PV infectious materials (IM) and potentially infectious materials (PIM)

The Islamic Republic of Iran has nominated a national authority for containment (NAC) and designated two polio essential facilities (PEFs): Razi Institute and Institute Pasteur. Only the Razi Institute has applied for a certificate of participation (CP), but this is pending clarification of queries raised by the Containment Secretariate at WHO headquarters. There is a dire need of auditors, and online training for auditors was provided to participants from the Islamic Republic of Iran. Pakistan has dissolved its NAC and a new NAC nomination is awaited, as is Pakistan's submission of a CP application for a serology laboratory as a PEF.

3. Recommendations

Polio laboratory management

1. NPLs should develop and implement contingency plans and share them with national authorities to integrate into national preparedness and outbreak response by the second quarter of 2022.
2. In anticipation of a global shortage of supplies, NPLs should keep the laboratory supplies inventory updated and share with the Regional Coordinator, with comments about critical items needed.
3. Annual laboratory supplies should be ordered in early 2022 and directors should exert efforts to find local suppliers for timely procurement.
4. WHO should maintain and top-up laboratory supplies at the Dubai Hub.
5. In the global polio laboratory network management system (GPLNMS), annual reports must be reported by end of February each year. Laboratory directors should frequently consult resources in GPLNMS and share updates with the staff.
6. Shipment of isolates and FTA cards to global specialized laboratories (GSLs) should be streamlined by identifying courier and appropriate referral GSL, due to limited flights and country restrictions from and to GSLs. Each laboratory should share the

outcomes of this exercise with the Regional Coordinator by end of the first quarter of 2022.

7. Laboratories should update standard operating procedures (SOPs) and quality assurance (QA) documents to include nOPV2 detection, characterization and management, including novel and modified assays, training records, storage, reporting and referral procedures.

Environmental surveillance system

8. WHO should support priority countries for the initiation and expansion of ES and monitor and review ES sensitivity and functionality.
9. ES laboratories should support the country ES system through regular communication and by providing advice on ES site performance, based on laboratory indicators and the local epidemiological situation.
10. All ES laboratories should ensure that staff are adequately trained and the WHO-recommended ES testing algorithm is consistently used. Any change in the processing of ES samples should be communicated to the Regional Coordinator.
11. Specific attention should be given to biosafety procedures during ES sample processing and documented for biorisk management.
12. Between the end of 2021 and the beginning of 2022, ES laboratories will receive the proficiency test (PT) panel for testing. The panel will be graded and is part of the ES accreditation scheme. All ES laboratories should participate in the upcoming ES proficiency testing.

Direct detection and nOPV2 roll out

13. WHO in collaboration with CDC should provide training to regional priority laboratories for pilot testing of the new DD assays and for subsequent implementation of the method.

14. WHO should keep the network laboratories updated on the progress of pilot testing for DD of poliovirus.
15. In anticipation of the DD roll out, all laboratories should conduct a comprehensive review of their molecular capacities, including infrastructure equipment and staff, and share any foreseen challenges with the Regional Coordinator.
16. All laboratories should be kept updated about the use of the nOPV2 algorithm for detection of nOPV2 strains.
17. Laboratories should have sufficient stock and will be timely provided through CDC/International Reagent Resource (IRR) with ample quantities of kits.
18. Laboratories should sequence all PV2 isolates, including VP1, in the context of nOPV2 use.
19. Laboratories should be given clear instructions on what PV2 isolates to refer to a GSL for whole-genome sequencing and incorporate this information in their SOPs and documentation. This might include both nOPV2+ and nOPV2- PV2 isolates from AFP and ES surveillance.
20. Special attention should be given to cell culture procedures to maximize the virus titres achieved on RD passage that will have an impact on the ability to generate whole-genome sequences from spotted FTA cards.
21. All EPLN laboratories should ensure:
 - mycoplasma-free cell cultures are used;
 - clear CPE is present before collection of virus-positive cultures;
 - cells are sensitive for poliovirus detection; and
 - appropriate methods are used for spotting virus cultures on FTA cards along with card storage and shipment procedures.
22. The ES PT panel in 2021 also contains nOPV2 and will be received by all ES laboratories. nOPV2 detected in ITD testing should be reported and referred for whole genome sequencing and shipped to GSLs.

ITD and VDPV testing quality assurance

23. All laboratories should register with IRR for PT panels and diagnostic kits. For the latter, laboratories should make an inventory of their current stock and order for 2022 depending on their workload and the version of kits on hand for supplementing.
24. ITD laboratories should take note of the new criteria for PT panel scoring and may consult the Regional Coordinator and CDC for their queries. The target is to have all laboratories participating in the 2021 PT panel.
25. WHO should continue to support ongoing quality assurance procedures for Poliovirus ITD and VDPV assay.
26. Low workload laboratories should continue to run at least one ITD and VDPV reaction per month to maintain competency and quality assessment of ITD equipment and reagents. This should be documented and shared with the Regional Coordinator.

Polio sequencing

27. CDC should consider working with WHO to provide a guidance paper for use of primers to troubleshoot the sequencing of difficult viruses.
28. All polio sequencing laboratories pending their accreditation should expedite testing of the PT panel.
29. CDC should organize workshops on sequence analysis and VDPV classification and reporting.
30. Training workshops should include genetic analysis principles and supporting bioinformatics software and tools.
31. The WHO Regional Office should monitor outcomes for the upcoming pilot testing of different sequencing procedures and plan for implementation in 2022–2023, as relevant and needed.

32. Laboratories with an interest in using the MinION system for sequencing PV isolates should contact WHO and GSL for advice and training options.
33. VACSERA and CDC should write a paper on troubleshooting for the sequencing of viruses spotted on FTA cards.

Primary immune deficiency surveillance

34. Laboratories should support PID surveillance implementation and integrate into AFP surveillance. In this regard, the laboratory should coordinate with the national PID programme and focal point in the ministry of health and at WHO.
35. Any support needed for this activity should be identified and shared with the Regional Coordinator.

Polio transition and integration

36. Considering the public health emergency situation, the heads of laboratories, in collaboration with ministries of health and national authorities and stakeholders, should work with WHO to ensure:
 - identification of alternative resources for sustaining laboratory functions at certification standards and addressing the potential reduction in GPEI resources;
 - integration of existing polio assets in other relevant public health priority areas by working with national vaccine preventable disease surveillance and health emergency units; and
 - prioritization of integration of existing polio assets in other relevant public health priority areas.
37. All laboratories should comprehensively document and share with the WHO Regional Office and WHO headquarters, all activities

undertaken with other public health programmes to help monitor and evaluate progress, transition and integration.

Containment and implementation of GAPIII

38. EPLN laboratories should support the National Polio Containment Coordinator to enter and update the national poliovirus and potentially infectious materials inventory in the web-based containment database management system.
39. All network laboratories should make use of the risk assessment tool which has been developed based on the GAPIII's annex 6 and is available in the containment database management system. Laboratories may plan mitigation measures according to analysis results.



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