Summary report on the

Twentieth intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region WHO-EM/POL/439/E

Tunis, Tunisia 22–24 October 2018



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1. Introduction

The twentieth intercountry meeting of directors of national and regional poliovirus laboratories in the Eastern Mediterranean Region was held in Tunis, Tunisia, on 22–24 October 2018. The meeting was attended by directors of national poliovirus laboratories from 12 countries of the Region, members of the Eastern Mediterranean Regional Certification Commission and national certification committees, and technical experts from: Centers for Disease Control and Prevention (CDC), United States of America; Kenya Medical Research Institute (KEMRI), Kenya; and the National Polio Laboratory, Turkey. World Health Organization (WHO) staff from headquarters, country offices and the Regional Office for the Eastern Mediterranean also attended.

The objectives of the meeting were to:

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

Dr Yves Souteyrand, WHO Representative to Tunisia, delivered a message on behalf of Dr Ahmed Al-Mandhari, WHO Regional Director for the Eastern Mediterranean, praising the high level of commitment and progress made towards the eradication of poliomyelitis in the Region. He commended the contribution of the Eastern Mediterranean Region Polio Laboratory Network (EPLN) in support of surveillance activities and early detection of polioviruses for a timely response in the field. He also commended countries for maintaining high surveillance standards and highlighted the strategies adopted to eradicate wild

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poliovirus (WPV) transmission in the two remaining endemic countries, Afghanistan and Pakistan, as well as the action taken to supplement acute flaccid paralysis (AFP) surveillance with environmental surveillance in at-risk countries and maintain the highly efficient environmental surveillance in Afghanistan, Egypt and Pakistan, and the progress made towards completion of Phase 1 of GAPIII for the containment of polioviruses and potential infectious material. He encouraged the EPLN to continue to support AFP surveillance for polio eradication activities.

The meeting reviewed the points of action from the previous intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region, and expressed satisfaction with their implementation. Those points of action that had not been fully implemented were repeated, including those on: the preparation of contingency planning; simulation exercises to evaluate the level of readiness in emergency situations; low workload laboratories' performance of real-time reverse transcription polymerase chain reaction (rRT-PCR) intratypic differentiation (ITD) on known viruses; and participation in exercises for the interpretation of rRT-PCR ITD run files.

2. Summary of discussions

Overview on the progress of polio eradication in the Region

All EPLN laboratories have maintained certification standard performance indicators and the well-organized EPLN has strengthened its diagnostic capacities. The Syrian Arab Republic national poliovirus laboratory has implemented poliovirus ITD methods. As part of the environmental surveillance expansion plan, new environmental surveillance laboratories have been established and are fully functional in Islamic Republic of Iran, Sudan and Syrian Arab Republic. Iraqi laboratory staff have been trained in poliovirus ITD

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testing and environmental surveillance laboratory testing methods, and these are expected to be functional as soon as the laboratory space has been renovated. To enhance capacity and self-sufficiency in poliovirus genome sequencing in Egypt, Islamic Republic of Iran and Oman, national poliovirus laboratories have been supported to establish genome sequencing methods, with the support of CDC.

In the Eastern Mediterranean Region, as of October 2018, there were 22 wild poliovirus type 1 (WPV1) cases, five AFP cases of circulating vaccine-derived poliovirus type 2 (cVDPV2), seven AFP cases of circulating vaccine-derived poliovirus type 3 (cVDPV3), 135 WPV1 isolates from environmental surveillance, 13 cVDPV2 isolates from environmental surveillance, and 10 cVDPV3 isolates from environmental surveillance (including one case with co-infection of cVDPV2 and cVDPV3). In Syrian Arab Republic, there have been no cases since September 2017, and an outbreak response assessment concluded that transmission of cVDPV2 has been interrupted, but cautioned about the high likelihood of new developments due to a weak vaccination programme.

Overall, the epidemiological situation shows that endemic circulation continues in a few areas of Afghanistan and Pakistan, the outbreak of cVDPV2 and cVDPV3 in Somalia continues with evidence of spread to Kenya, and the risk of spread in Horn of Africa countries is imminent (with one isolate in Kenya), and therefore an outbreak response is being implemented in the entire Horn of Africa.

As regards to progress in polio eradication during 2017–2018, national emergency action plans are being implemented in Afghanistan and Pakistan, a multi-country response to the cVDPV2 and cVDPV3 outbreak in Somalia is being implemented, and surveillance performance indicators have been maintained at or above certification standard in 20

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out of 22 countries. Quarterly risk assessments, desk reviews of prioritized countries, and field reviews are performed regularly.

Challenges to polio eradication include being able to reach: all children for immunization in the conflict-affected parts of Afghanistan, Libya, Somalia, Sudan, Syrian Arab Republic, and Yemen; mobile groups in Afghanistan, Pakistan, Libya, Somalia, Sudan, and Yemen; and internally-displaced populations and refugees in Jordan, Lebanon, and Syrian Arab Republic. Another obstacle is the damaged health infrastructure in Iraq, Syrian Arab Republic and Yemen. Other challenges include maintaining high population immunity and surveillance performances at subnational levels where these have been achieved, maintaining political and community engagement, and sustaining adequate resources.

Global polio eradication status and overview

As of October 2018, the Global Polio Eradication Initiative (GPEI) reported the lowest number ever (22) of WPV1 cases globally, with no WPV3 case reported worldwide since November 2012. cVDPV outbreaks have been reported in the Horn of Africa and three different cVDPV2 outbreaks have been detected in AFP cases in the Democratic Republic of the Congo, declared a national public health emergency. A cVDPV2 outbreak has been confirmed in Niger due to inaccessibility in Borno state and large-scale population movement within and across countries; the outbreak is related to cVDPV2 in Jigawa, Nigeria. Papua New Guinea confirmed a polio outbreak due to VDPV1, with 18 VDPV1 cases and four positive environmental samples reported as of 25 April 2018. The detection of cVDPV underscores the importance of maintaining high levels of routine vaccination coverage at all levels to minimize the risk and consequences of any poliovirus circulation.

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As the world gets closer to the goal of polio eradication, WHO, along with other partners of the GPEI, is planning for the transition of the assets and capacities of the polio programme and eventual closure of the programme. A strategic action plan on polio transition has been developed with three main objectives: sustaining a polio-free world; strengthening immunization, including vaccine-preventable diseases surveillance; and strengthening emergency preparedness, detection and response capacity to fully implement the International Health Regulations (2005).

Laboratory network performance and challenges dealing with the workload

The quality of laboratory performance indicators for EPLN laboratories has been maintained at a high standard despite the numerous difficulties and challenges, including war, conflict and insecurity. All poliovirus laboratories in the Region are fully accredited. Despite the high workload, laboratories in Egypt and Pakistan are maintaining the timeliness and accuracy of their results.

regional reference laboratory in Pakistan The serves as a national/regional laboratory for both Afghanistan and Pakistan. It has tested more than 30 000 stool samples from AFP cases and their contacts in both countries. It is the only laboratory in the Region that has the capacity to test poliovirus serology in the community and is supporting the polio eradication programme. In addition, the laboratory is involved in pilot testing of a new environmental sampling tool, the bag-mediated filtration system (BMFS), developed by PATH, USA and University of Washington, USA. Both Afghanistan and Pakistan have expanded their environment surveillance network in 20 and 57 sites, respectively.

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The regional reference laboratory in Egypt has maintained excellent performance over many years. The number of samples for testing has increased due to increased samples collected from contacts of hot AFP cases, inadequate cases and VDPV cases. The laboratory has tested around 3500 stool samples received through AFP surveillance and the primary immune deficiency disorders (PID) project. The laboratory supports the polio eradication initiative and is providing ITD and genome sequencing testing facilities for Iraq, Jordan, Lebanon, Sudan, and Syrian Arab Republic. In order to minimize the impact of an increasing workload, the laboratory has established separate areas of work for handling and clear lines of responsibility. The laboratory supported the testing of poliovirus-positive samples during the cVDPV2 outbreak in Syrian Arab Republic. In addition, the laboratory is testing 45 samples per month from 27 governorates, and testing stool samples from immune-deficient individuals as a part of a regional study.

The KEMRI poliovirus laboratory in Kenya is supporting both AFP and environmental surveillance laboratory testing of samples during the ongoing cVDPV2/3 outbreak. Their support to countries of the Region is highly appreciated.

Progress towards detection and interruption of WPV and VDPV

As of 15 October 2018, 22 paralytic poliomyelitis cases due to WPV1 have been identified, including 16 paralytic cases from Afghanistan and six from Pakistan. Environmental surveillance in both countries has also confirmed the ongoing transmission of WPV1. Based on the genetic information, and given the challenges and concerns in both countries, the progress towards polio eradication is encouraging. However, while a sustained decline in genetic diversity with disappearing clusters and lineages is clear, AFP and environmental surveillance sampling indicate the persistence of WPV1 in three key

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reservoirs (the northern corridor, southern corridor and Karachi), which pose a risk to areas with low immunity, alongside the continuous risk of reseeding in non-reservoir areas.

Widespread circulation of WPV1 in the northern corridor has seeded other districts of Khyber Pakhtunkhwa and the northern Punjab. Genetic sequencing data from environmental isolates indicate that the WPV1 isolated from Pakistan has also been detected in Afghanistan. Genome analysis of the WPV1 indicates cross-border transmission.

The large outbreak of cVDPV2 detected in Syrian Arab Republic during 2017 has been successfully stopped with no international spread. This outbreak provided an opportunity to review preparedness capacity at the regional level and experience coordination efforts between the polio laboratory networks of three WHO regions, involving five different countries and laboratories.

Two simultaneous outbreaks of cVDPV2 and cVDPV3 have been reported. The first case was a co-infection by cVDPV2 and cVDPV3 detected in Bulo Burti district, Hiran province (date of onset 11 May 2018); cVDPV2 was first isolated from an environmental surveillance sample collected on 4 January 2018 from Hamar Weyn district, Banadir province, and cVDPV3 was first detected in a sample collected on 11 February 2018 also from Hamar Weyn district. These cVDPV cases have no genetic links to any past cVDPV2 and type 3 outbreaks.

As of October 2018, Somalia had reported 12 cases due to cVDPV, including five due to cVDPV2, six due to cVDPV3 and one due to co-infection by cVDPV2 and cVDPV3. Environmental surveillance isolated cVDPV2 from 19 sewage samples in Mogadishu, with one of the 19 samples having co-infection by cVDPV2 and cVDPV3;

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cVDPV3 was isolated from 12 sewage samples in Mogadishu, and one of these 12 had co-infection by cVDPV2 and cVDPV3.

Primary immunodeficiency disorder (PID) surveillance and immunodeficiency-related vaccine-derived polioviruses (iVDPVs)

PID 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 systematically integrate PID in 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. From 2007 to 2017, stool samples from 607 PID patients were tested. The study will help to provide guidelines for future implementation of PID surveillance in other countries.

A laboratory in the Islamic Republic of Iran has tested 545 specimens from 262 PID cases as part of the project, detecting only seven iVDPVs so far. One AFP case has excreted iVDPV1 for ~six months (six specimens received so far). One case of iVDPV2 has been excreting the virus for more than three years and is being treated with pocapavir.

In Egypt, 297 PID patients were tested from 2011 to 2018, with 13 iVDPV cases detected. The duration of virus shedding is less than six months, except one case, which is a prolonged excretor. All patients stopped iVDPV excretion after treatment with intravenous immunoglobin. The project is being continued and expanded to better understand the risks iVDPV exectators pose to global poliovirus eradication.

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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. CDC is continuously updating protocols and algorithms for poliovirus molecular testing for better testing sensitivity and specificity with rapid virus detection and sequencing during outbreaks. To avoid lengthy methods, a new algorithm for poliovirus detection and identification is needed that is highly sensitive, robust, simple and costeffective. Two newly designed poliovirus direct detection protocols from stool samples, the poliovirus receptor (PVR)-His-tag method and Zymo column double extraction method, are being tested and compared with the current virus isolation algorithm. The results are quite promising, with pilot testing showing that both direct detection methods are significantly more sensitive than the virus isolation algorithm. Parallel testing with the virus isolation algorithm and the double extraction direct detection protocol is in progress.

Furthermore, CDC is updating the ITD rRT-PCR protocol, with version 5.0 updated to version 5.1, which is almost identical to version 5.0. A major change is the new PanPV assay (Zen8-panPV probe) that helps to increase sensitivity and resolve issues such as the presence of weaker signals, especially on Rotor-Gene, and atypical results.

Maintaining laboratory quality assurance

Based on annual laboratory performance, WHO has recently reviewed virus isolation, ITD, and sequencing accreditation checklists. Revisions proposed for virus isolation include those on safe handling and storage of poliovirus type 2, biosafety practices and procedures, and updating the protocol for cell sensitivity testing to make it

compatible as per requirement. Annual accreditation of environmental surveillance laboratories has been initiated, based on a checklist for quality indicators and laboratory systems developed for environmental surveillance for polio eradication. In addition, an environmental surveillance proficiency test panel has been prepared and is currently being evaluated in two laboratories. The virus isolation proficiency test panel will now be shipped directly from the Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

The annual proficiency test is being continued for four different proficiency testing panels: virus isolation accuracy; ITD by rRT-PCR; rRT-PCR for VDPV screening; and sequencing poliovirus isolates.

The Syrian Arab Republic laboratory started ITD testing in May 2018, and is awaiting the results of proficiency test panels for final accreditation as an ITD laboratory.

The issues and challenges for AFP surveillance encountered by the Iraqi national polio laboratory in 2018 include problems of preventive maintenance and biorisk management, turnover of staff due to work overload, inadequate space, and financial limitations.

Oman's poliovirus sequencing laboratory has just been established, and a proficiency test panel was performed in 2017, with the results submitted to CDC, and feedback indicating that the run sequences were identical to the reference Sabin strain.

The VACSERA laboratory in Egypt has carried out four types of trial session and is now confident in sequencing poorly-amplified samples and has overcome problems of incomplete coverage of VP1, contigs with opposing directions, and poor sequence quality. The cumulative experiences from all trials led to a 100% score in the last sequencing

proficiency test panel. Now the laboratory has full capacity to sequence isolates with discordant results and poliovirus type 2.

When results/information are entered for all samples in the Global Polio Laboratory Network Management System, laboratories will now be offered the possibility to upload documents. A pdf version of the laboratory's worksheets will also be uploaded with these results.

Environmental surveillance

Poliovirus environmental surveillance has been used to supplement AFP surveillance and help detect wild or VDPV circulation to guide surveillance and immunization activities. The GPEI has made tangible progress in implementing the global environmental surveillance expansion plan. So far, environmental surveillance for polioviruses has been implemented in 22 out of 34-targeted countries globally. In total, 153 sites have been selected in the Eastern Mediterranean Region, covering nine countries (87 cities): Afghanistan (20 active sites), Egypt (46 active sites), Islamic Republic of Iran (two active sites), Jordan (three active sites), Lebanon (four active sites), Pakistan (57 active sites), Sudan (three active sites), Somalia (five active sites), and Syrian Arab Republic (13 active sites). Expansion of environmental surveillance is planned in Iraq, Saudi Arabia and Yemen to monitor the high risk of poliovirus importation in these countries due to their geographical location and mass gatherings.

Pakistan's regional reference laboratory has pilot tested algorithms with different modifications to remove bacterial contamination during the processing of raw sewage samples. The study found satisfactory results were obtained with the introduction of a high concentration of antibiotics added to the maintenance media to control bacterial

contamination. A newly modified protocol has been shared with all poliovirus environmental surveillance laboratories in the Region.

The Environmental Surveillance Implementation Working Group is working on performance indicators, particularly in relation to certification of global polio eradication and protocol development for environmental surveillance in an outbreak setting, to fine tune sampling sites. These indicators will monitor the completeness and timeliness of field and laboratory processes.

Poliovirus serology testing

Pakistan's regional reference laboratory is the only polio serology facility in the Region, and has high testing throughput, capacity and automation. A first polio sero-survey was conducted in Pakistan from January 2017–April 2018. Site selection was based on poliovirus epidemiology during 2013–2014 and a total of 16496 serum samples were tested.

From June–December 2018, the polio serology laboratory in Pakistan is undertaking a Phase 3 polio sero-survey in collaboration with Aga Khan University, testing 6000 serum samples from 18 districts in Pakistan to support a national level assessment of population immunity and strengthen eradication efforts. The facility can be utilized to support similar large-scale/nationwide surveys due to its high throughput analytical capacity. It can provide an annual immunity assessment of polio laboratory staff in Pakistan and, in coordination with WHO, in other regional laboratories.

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Containment of polioviruses in poliovirus laboratories

In terms of the implementation of GAPIII for the containment of polioviruses, the destruction of WPV2/VDPV2 material has been completed in all facilities of the Region, while inventories of potentially infectious material are in progress, and efforts are being made to complete Phase 1 of GAPIII by April 2019. WHO guidance to minimize the risks for facilities collecting, handling or storing potentially infectious materials for polioviruses was revised during April 2018.

Islamic Republic of Iran and Pakistan have designated polio essential facilities and nominated a national authority for containment. Lack of appropriate auditors to audit polio essential facilities seeking the certificate of containment is a challenge in the Region. In response, WHO is planning to train auditors from Islamic Republic of Iran and Pakistan.

3. Points of action

Coordination between polio laboratories and related public health programmes

- As part of transition planning, polio laboratories should map their assets and functions, and share this information with national health authorities, in order to identify common processes and interests to help strengthen cooperation with other public health programmes, including surveillance for vaccine-preventable diseases, such as measles and rotavirus, and other communicable diseases.
- The unique experience of coordinating efforts between polio laboratories in different regions during investigation of the cVDPV2 outbreak in Syrian Arab Republic should be documented.

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Contingency planning to deal with an unforeseen increase in workload

• To respond to unforeseen increases in workloads due to the expansion of activities, a polio outbreak, equipment failure, personal issues, and/or non-polio emergencies, regional laboratories need to develop a contingency plan in coordination with the WHO regional coordinator and the Expanded Programme on Immunization/Ministry of Health. WHO will share a contingency plan template, which should be institutionalized and integrated into national preparedness and outbreak response plans.

Simulation exercise for laboratory planning and preparedness

- Polio laboratory is currently only a small part of the polio outbreak simulation exercise. WHO should develop and implement polio laboratory simulation exercises to evaluate the level of readiness in emergency situations.
- A module for polio laboratory simulation exercises should be developed by WHO, including discussions on laboratory methods, results interpretation and reporting. This module may be included in national polio outbreak simulation exercises.

ITD and sequencing performing laboratories

- Low workload laboratories should devise a plan for maintaining their ability to perform WHO-accredited laboratory assays:
 - laboratories should regularly perform complete rRT-PCR ITD/sequencing tests and analysis on at least five known samples every month and share data with reference laboratories;
 - laboratories with a Rotor-Gene machine should use the ITD 5.1 kit; and

- laboratories should regularly analyse result files from rRT-PCR ITD tests, at least once every month, and share the data with reference laboratories.
- The Region should continue to expand its data bank of rRT-PCR ITD test result files for access by laboratories (through the reference laboratory/regional coordinator); the results of these activities should be reviewed and corrective action taken. Reviewers may use this during accreditation visits.
- The data bank will be shared with WHO headquarters.
- Laboratories in Egypt, Islamic Republic of Iran and Oman should continue implementation of WHO sequencing methods, sharing the results for assessment by CDC experts.
- Iraq's laboratory should expedite the establishment and functionalization of an ITD laboratory.

Quality assurance

- Laboratories should continue to participate in proficiency panel testing.
- Based on accreditation recommendations, a plan should be developed by the laboratory director and shared with the regional coordinator for follow-up.
- Pilot testing of environmental surveillance proficiency testing panels should be done in polio network laboratories.
- A sequencing proficiency test panel should be distributed to Iranian and Omani laboratories, pending WHO accreditation as sequencing laboratories.
- To sustain performance indicators for certification standards, laboratories should:
 - review the accreditation checklist as a pre-audit of their performance before accreditation review and as a tool for internal audit at least once a year;

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- revise and update standard operating procedures and worksheets, accordingly;
- strengthen quality assurance using the Laboratory Quality Management System and applying its essential elements; and
- use laboratory assessment tools to identify gaps and deficiencies, where necessary.

Direct detection of poliovirus from stool samples

- WHO should keep the EPLN updated on the development of new methods for direct detection.
- Regional reference laboratories may be asked to participate in pilot testing of a new algorithm.

Data management

- A web-based laboratory information for action data system should be developed in collaboration with WHO headquarters and the WHO Regional Office for Africa.
- A logistics support system should be distributed to laboratories to manage polio laboratory supplies.
- The Global Polio Laboratory Network Management System should be utilized in full. In particular:
 - it should be accessed and updated regularly;
 - virus isolation proficiency test data should be entered after testing results;
 - the accreditation checklist should be used for review;
 - annual reports should be completed before mid-February every year; and
 - each laboratory should develop a generic laboratory account and access should be granted to responsible staff.

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Transportation and reverse cold chain monitoring

- In countries/areas with suspected reverse cold chain issues, LogTag use may be indicated to improve quality. However, necessary steps should be taken to:
 - coordinate with the Expended Programme on Immunization for implementation;
 - share data analysis with field staff;
 - develop standard operating procedures; and
 - provide training to staff (field and laboratory).

PID surveillance

- Islamic Republic of Iran, Egypt and Tunisia should continue with the PID surveillance project and work towards its integration with the AFP surveillance system to enhance sensitivity for iVDPV detection.
- Countries opting to establish PID surveillance should follow WHO guidelines for implementation of PID surveillance.

Environmental surveillance

- Environmental surveillance data should be utilized to monitor environmental surveillance site selection and performance.
- Environmental surveillance laboratories should continue to provide their opinion on environmental surveillance site performance based on laboratory findings (rate of non-polio enterovirus and negative samples) to guide the programme onsite.
- In countries at risk of importation of WPV and/or emergence of VDPV cases, especially Iraq and Yemen, efforts should be urgently made for the establishment of environmental surveillance.

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- WHO should share the amended environmental surveillance procedure protocol (adding gentamycin) for use in environmental surveillance laboratories.
- Environmental surveillance laboratories should consider the use of a polytetrafluoroethylene Teflon funnel instead of a glass funnel to reduce biorisk.

Poliovirus containment and implementation of GAPIII

- Poliovirus laboratories, being an essential part of polio containment, should support and continue containment activities to complete Phase 1 of GAPIII by end April 2019.
- Laboratories should take risk assessment and mitigation measures, using WHO guidance, to minimize the risks for facilities in collecting, handling, or storing materials potentially infectious for polioviruses.
- Laboratories should support the national authority for containment by providing technical support for the certification of polioessential facilities.

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