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# Meeting of Regional and Global Specialized Polio Reference Laboratories of the WHO European Polio Laboratory Network

11–12 March 2015
National Institute for Biological Standards and Control, Potters Bar, United Kingdom

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## ABSTRACT

The main objective of the meeting was to make a final decision on the adoption of an updated algorithm for poliovirus isolation which is now used by GPLN laboratories in all other WHO regions. Adopting the new testing algorithm will help ensure that laboratories use fully standardized methods across the Region and will improve timeliness of detection of programmatically important polioviruses. The possible implications of using the new testing format and plans for its implementation were discussed at length based on the experience in some laboratories that adopted the change and responses to a comprehensive survey sent to all laboratories by the WHO Regional Office for Europe. The performance of the Polio Laboratory Network in the European Region and the importance of laboratory containment as required by WHO guidelines were also discussed. The meeting produced a set of recommendations for further action and consideration.



### Keywords

LABORATORIES
MEETING REPORTS
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POLIOVIRUS

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## Abbreviations

| | |
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| bOPV | bivalent oral polio vaccine |
| cVDPVs | circulating vaccine-derived polioviruses |
| CDC | United States Centers for Disease Control and Prevention |
| GAP III | 3rd Edition of the Global Action Plan for Poliovirus Containment |
| GLC | Global Laboratory Coordinator |
| GPEI | Global Polio Eradication Initiative |
| GPLN | WHO Global Polio Laboratory Network |
| ITD | intratypic differentiation |
| LDMS | laboratory data management system |
| NLC | National Laboratory Coordinator |
| NRAcs | national regulatory authorities responsible for containment |
| NIBSC | National Institute for Biological Standards and Control |
| OPV | oral polio vaccine |
| PT | proficiency testing |
| RLC | Regional Laboratory Coordinator |
| tOPV | trivalent OPV |
| VDPV | vaccine-derived poliovirus |
| WPV | wild poliovirus |

## Introduction

The meeting of regional and global specialized polio reference laboratories in the European Region of the WHO Global Polio Laboratory Network (GPLN) was held at the National Institute for Biological Standards and Control (NIBSC), Potters Bar, United Kingdom. The meeting included representatives from eight member laboratories, United States Centers for Disease Control and Prevention (CDC) and WHO headquarters and regional offices. A representative from the IT company Novel-t Innovative Solutions working with WHO in the development of an online-based laboratory management system also attended the meeting.

The main objective of the meeting was to make a final decision on the adoption of an updated algorithm for poliovirus isolation which is now used by GPLN laboratories in all other WHO regions. Adopting the new testing algorithm will help ensure that laboratories use fully standardized methods across the Region and will improve timeliness of detection of programmatically important polioviruses. The possible implications of using the new testing format and plans for its implementation were discussed at length based on the experience in some laboratories that adopted the change and responses to a comprehensive survey sent to all laboratories by the WHO Regional Office for Europe. The survey covered different aspects of laboratory work that could be affected by the switch including infrastructure, technical expertise, and national regulations. The performance of the Polio Laboratory Network in the European Region was also discussed by reviewing recent proficiency testing (PT) results in the various laboratory techniques used by the laboratories. Performance in the Region continues to be high although some concerns remain particularly in terms of the quality of reporting and the quality assurance of cell culture work. Challenges to improve timeliness of detection of wild poliovirus (WPV) and vaccine-derived poliovirus (VDPV) continue as international shipment of materials and reagents, such as virus panels, cells and clinical samples, is becoming increasingly difficult and expensive due to strict regulations in most countries. No paralytic cases due to WPV were reported during 2013–2014, but type 1 WPV was isolated from sewage samples in Israel between February 2013 and March 2014. The large number of WPV1 isolates found in many different locations was evidence of widespread circulation of WPV1 in a country with more than 95% coverage with inactivated polio vaccine. As a consequence, live-attenuated oral polio vaccine (OPV) was re-introduced in the country for routine use, which quickly resulted in the interruption of WPV1 circulation.

A representative from CDC presented an update in polio diagnostic protocols with special focus on molecular intratypic differentiation (ITD) and sequencing procedures and the future need to adapt virus isolation and ITD algorithms to changing priorities defined by the Endgame Strategic Plan for the Global Polio Eradication Initiative (GPEI). Special attention in the discussions was given to the importance of detecting and identifying circulating VDPVs (cVDPVs) as the presence/absence of these viruses will determine when changes in immunization strategies can be implemented. Efforts to develop improved laboratory methods and standardized quality assurance procedures continue to be a priority of the GPLN.

The importance of laboratory containment as required by WHO guidelines 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.

The group concluded that some of the aspects reviewed during the meeting should be thoroughly discussed during the coming all-laboratory meeting in September particularly those concerning the adoption of the new algorithm for virus isolation, with details of testing procedures, documentation and PT, and the implications of the GAP III for European laboratories.

## Summary of discussions

### *Global Polio Laboratory Network Management System*

An online management system has been devised by Novel-t Innovative Solutions in consultation with WHO that allows laboratories to produce annual reports by entering and updating laboratory data, details of laboratory capacities and laboratory indicator values. The system will also be used to generate accreditation reports that can be reviewed during the accreditation process. The use of this online system will improve coordination between the different levels of the GPLN by identifying and correcting gaps in the global management of information. It will be possible to capture comprehensive information relative to laboratory data generated by the GPLN and to archive all this information for easy access. It will also streamline key processes used to monitor the quality of laboratory performance such as annual reporting, accreditation and PT. Once a year, each laboratory is required to submit an annual report via the GPLN platform. The report provides a snapshot of a laboratory and its capacities at the time of submission. All

three levels of laboratory coordination (national, regional and global) are involved in the production and submission of the annual report. The process is entirely managed on the online platform where the status of the report changes depending on the actions taken by the different elements involved in the process. To initiate the process, the Regional Laboratory Coordinator (RLC) or Global Laboratory Coordinator (GLC) sends an email to the National Laboratory Coordinator (NLC), indicating the timeline for the reporting process. The submission deadline usually occurs towards the beginning of a calendar year. Firstly, the NLC enters the data into the report and submits it. The RLC reviews the report and approves it following revisions if necessary. Once approved, it is forwarded to the GLC who validates it after any necessary modification. Once the report is validated, the annual report process is finalized and archived. In order to produce the annual report, two types of laboratory data need to have been entered by the NLC in the platform:

1) Laboratory data

These data consist of general information about the laboratory (e.g. shipments, staff and equipment) or information related to the laboratory's capacities (e.g. on-site reviews). This information can be entered at any time during the year as long as it is up to date when the annual report is submitted. The information in the different tabs is automatically copied into the annual report at the moment of its submission.

2) Laboratory indicator values

These data are linked to the capacities of a laboratory and are entered in the platform during the annual report submission process. This information can only be entered once the annual report process has been initiated.

The system will also be used for accreditation as the accreditor will have access to the accreditation report prepared by the laboratory using the system. The workflow in this case depends on the laboratory type. Firstly, the laboratory will prepare and submit the accreditation report. The accreditation report consists of three different checklists, one per capacity. Permanent information is automatically included by the system in each checklist. The accreditor reviews the report and chooses either to return it for amendment or to approve it for evaluation by the RLC. The RLC reviews the report and in the case of NLC chooses either to reject the report and send it back for revision or to accept it. In the case of regional and global specialized laboratories the RLC validates the accreditation report and sends it to the GLC for final review. Finally, the GLC reviews the report and accepts it following any necessary amendment. Once the report is

accepted, it is archived and accreditation status is set. The annual report module is being tested by 20 laboratories that will provide feedback. A few laboratories from all regions to be accredited in 2015 covering all capacities will be selected to beta test the electronic process for accreditation. There are also plans to compute annual and accreditation indicators from laboratory files received at WHO POLIS in order to verify the values reported by the laboratories through the online system. The system will be expanded to include electronic support for virus isolation PT and to allow sharing documents, discussion forums, access to FAQs, newsletters, publications, etc. More information about the system and access to a demo can be found here: https://extranet.who.int/gpln-uat

### *ITD 4.0 assay*

The only ITD method that is now supported by WHO is that based on real-time RT-PCR (rRT-PCR) developed by CDC. One of the possible consequences of changing to the new virus isolation testing algorithm in the European Region will be that more laboratories will be willing to implement this ITD method as it will allow them to improve the diagnosis of virus isolates from clinical samples. 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 also be routinely used for the characterization of poliovirus isolates from environmental samples and eventually for the direct detection of poliovirus isolates from stool samples if such a method is finally validated for use among GPLN laboratories. Laboratories in the European Region have contributed to the pilot-testing and validation of rRT-PCR ITD assay 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 also been tested and found to increase the sensitivity for poliovirus detection especially in samples containing mixtures. Work still remains to validate VDPV rRT-PCR rule-in assays. The type 2 assay is usable now but some caveats remain for type 1 and 3 tests. The new ITD 4.0 algorithm has fewer discordant results due to not using serotype assays which may fail in mixtures. Further improvements were also included such as the CODEHOP Pan-PV assay, optimizing probe concentrations to reduce background and redesigning WPV and Sabin 1 probes to eliminate cross-reactivity. Implementation plans for the

rRT-PCR ITD 4.0 test have started. Kits will include 100 reactions instead of 50 as in previous kits. As they include fewer components they are easier, cheaper and faster to produce. Kits with EV/Sabin quadruplex, PanPV, WPV1 duplex and WPV3 assays are ready to ship. VDPV kits are in production so laboratories can continue to use current kits for VPDVs. It is recommended that the test is adopted as soon as possible in high-workload and/or laboratories that have pilot-tested the method. These laboratories will need minimal or no training. The test will be phased in in other laboratories during 2015. The rule-in VDPV2 assay might be added in late 2015.

### *Detection and identification of VDPVs*

With the significant decline in poliomyelitis cases due to WPV in recent years, cases due to VDPVs assume greater importance. Effectively detecting cVDPVs will be critical during the Endgame of GPEI. The trigger for setting up a date for the withdrawal of type 2 live-attenuated OPV from the trivalent OPV (tOPV) which is planned for as early as 2016, will be determined by the absence of persistent cVDPV2 for at least six months. This means that using sensitive and effective surveillance systems for detecting and classifying poliovirus isolates as VDPVs will be required. The current rRT-PCR VDPV assay has some limitations as it identifies viruses that match Sabin 2 in the target region so in homotypic mixtures containing Sabin 2 and VDPV2 viruses, the Sabin 2 signal can mask the presence of a VDPV2 isolate. Furthermore, up to 50% of the Sabin 2 isolates found in Nigeria might not match Sabin 2 in the target region even though they are ordinary Sabin 2 viruses, and therefore many isolates must be sequenced using up resources and time. New improved methods are being developed such as a rule-in rRT-PCR VDPV tests and assays based on deep sequencing techniques, digital PCR, plaque purification prior to sequencing, etc. As tracking persistent cVDPVs is essential before changes in immunization can take place, obtaining accurate dates of VDPV emergence is critical. This might require analysing complete capsid or complete genome sequences to establish clear genetic links between virus isolates. The input of GPLN scientists in this process is critical. The availability of a VDPV sequence database including information on all VDPV strains sequenced to date would be very useful. Effective communication between GPLN scientists and epidemiologists is important as it will help decision-making on how to classify and date VDPV isolates. The programmatic relevance of VDPV isolates from immunodeficient individuals is not so clear although their presence as a possible source for polio re-emergence cannot be disregarded. A hypogammaglobulinaemic individual in the United Kingdom has been excreting type 2 VDPV for an estimated 28 years. This represents by far the longest period of excretion

described from such a patient. The virus is very virulent, antigenically drifted and excreted at high titre, suggesting that such chronic excretes pose an obvious risk to the eradication programme. Although this is the only identified individual known to be excreting highly evolved vaccine-derived poliovirus at present, several highly drifted VDPV strains have recently been isolated from sewage samples in Estonia, Finland, Israel and Slovakia. They included examples of all three poliovirus serotypes, although type 2 VDPVs were the most prevalent among them. These VDPV isolates showed molecular properties typical of VDPVs from immunodeficient individuals indicating that an unknown number of these chronic excreters exist elsewhere. Efforts are currently on-going to identify molecular markers/properties that clearly classify VDPVs found in environmental samples as circulating or from immunodeficient patients.

### *Laboratory quality assurance*

The annual PT and assessment of laboratories continue to be critical for the quality assurance of the performance in polio laboratories. Three different PT panels are in use for evaluating: a) accuracy of virus isolation; b) ITD by real-time PCR (rRT-PCR); c) sequencing poliovirus isolates. The PT programme is coordinated by WHO in collaboration with the global specialized laboratories in the United States and the Netherlands.

Laboratories in the European Region were given the option to conduct the 2014 PT for virus isolation using either the old or the new method. One laboratory chose to use the new algorithm alone, 28 used the old method and 7 a combination of both. Thirty four laboratories did the PT in 2014. Laboratories linked to the Regional Laboratory in Moscow will complete this test in 2015, as import permit issues prevented shipment in 2014. The two laboratories in Moscow have already reported results and others will follow soon as distribution of panels from the Regional Laboratory in Moscow has started. There were some errors in PT results from European laboratories, which included errors in reporting, serotyping and failing to detect specific cell infections. The most problematic sample was sample 10, which contained a mixture of type 1 poliovirus and echovirus 6. Three laboratories failed to pass the test. One laboratory reported L20B positive infection in sample 9, which only contained coxackievirus B1. The interpretation by the Global Specialized Laboratory in the Netherlands was that L20B cells used in this laboratory were likely contaminated with human cells leading to the unexpected result. This was confirmed at the Global Specialized Laboratory in the United Kingdom, which found that L20B cells from both master and working cell banks contained large proportions of cells of human

origin as found using the established RT-PCR test for cell authentication. The Regional Reference Laboratory in Rome after verification by the GSL, United Kingdom of the authenticity of their cells sent L20B master cells to the affected laboratory. The three laboratories that failed the PT passed a repeat test with 95% (mistyped non-polio enterovirus), 100% and 100% score respectively.

Extra tools for the quality assurance of cell culture laboratory work developed by the Global Specialized Laboratory in United Kingdom have been successfully introduced in GPLN laboratories. A standardized test to measure the sensitivity of the cell lines for poliovirus infection is available and regular testing is required for laboratories to be accredited. Many laboratories in the Region continue to have issues related to cell culture procedures, some of them with recurring problems. Common signs of actual problems with cell sensitivity for poliovirus infection include repeated low titres of cell sensitivity reference standards with respect to established titre values. In several cases, most problems with cell sensitivity testing appear to be mainly technical deficiencies in applying/interpreting the test as indicated by excessively high virus titres or repeated identical titre values which are statistically impossible. This situation has to be promptly addressed. Cell culture workshops are planned. A standard real-time PCR method for cell authentication of cell lines used in the laboratory has now been fully validated and is available for testing master and working cell banks from GPLN laboratories.

PT for rRT-PCR ITD and sequencing was also performed in European laboratories using these tests. Results for the 2013 PT for rRT-PCR ITD were excellent with all 6 laboratories achieving >90% score, with a minimum score of 95%, a maximum score of 100% and an average score of 98%. Minimum, maximum and average scores for the VDPV rRT-PCR reaction were 94.5%, 100% and 99.1%, respectively. The plan for 2014 was to increase the stringency of scoring similarly to what is done for the virus isolation PT, for which failure to identify/detect a WPV or VDPV results in the automatic deduction of 15 points and a failed score. There were other changes in the PT as samples for both rRT-PCR ITD and VDPV reactions were combined in a single panel and laboratories were asked to send a table of results and raw data to CDC within seven days of receiving the PT. The score was based on the final result and a score of ≥90% was needed to pass. There was a higher point loss for failure to detect WPV or VDPV in samples (-15%). Additional deductions were given for technical issues and a 5% deduction for each week of delayed reporting. Unfortunately, using the revised scoring system resulted in more than 50%

of laboratories globally failing the PT with only two out of the six laboratories in European passing the PT. The panels were finally scored using the previous system giving a 10% value to each sample and no partial credit for specific viruses. In these conditions all European laboratories passed the test. The scoring system will be reviewed after implementation of the ITD 4.0 test. There were some other challenges in the 2014 PT 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. Following review of past problems encountered in laboratories 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. European laboratories are highly proficient in polio PCR assays, turnaround time is excellent for all laboratories and PT results correlate with routine results. Interpretation remains the biggest issue throughout the GPLN.

The PT for sequencing requires the sequence analysis of PCR fragments containing the gene coding for VP1 capsid protein which is used to establish epidemiological links during surveillance essential to monitor the progress of polio eradication. RNA samples are sent to laboratories that are requested to amplify the VP1 region using generic or specific poliovirus primers to identify individual virus or components in a mixture. Laboratories should then sequence the amplified PCR products, edit the results using specialized software and provide a final nucleotide sequence result. Technology transfer from CDC including methods and reagents has been on-going for more than 20 years including training activities and onsite consultations. Standardized methods and kits containing amplification and sequencing primers that detect all known polioviruses are available from CDC. PT for sequencing was introduced in 2011 with no scoring given. In 2012, scoring was introduced and there was a 14-day time limit to send results. Four samples with individual virus RNAs were sent to laboratories. Sequencing virus mixtures was optional by analysis of an extra sample included in the PT panel. In 2013, more stringent requirements were introduced. Double-stranded sequence data were required throughout the VP1 gene and virus mixtures had to be sequenced. There were, however, some issues with the 2013 PT sequencing panel as unexpected problems in RNA concentration and stability were found in some samples. This was probably due to the use of a new pellet paint version not used in previous panels. As a consequence, only two samples were scored. All but two laboratories globally obtained a passing score. Four samples were again included in the 2014 panel. Five

European laboratories scored 100%, two laboratories scored between 90% and 99% and one laboratory obtained a score lower than 90%, failing the PT. A survey included with the PT process found that two laboratories were using the WHO RT-PCR protocol, 5 a one-step RT-PCR generic method and 1 did not specify. A variety of software packages was used for sequence analysis, which included Sequencher, Geneious, BioNumerics and CodonCode Aligner. The samples were sequenced using either in-house facilities, outside contractors or both. Remedies to support laboratories failing the sequencing PT were proposed. They will include follow-up by the Regional Laboratory Coordinator and scientists from global specialized laboratories to evaluate current practices and suggest possible improvements. Laboratories with low workload should sequence at least 25 isolates annually. Laboratories that failed the 2014 PT will repeat the PT during 2015. Possible refinements in the PT process were also discussed. They could include using FTA cards for RNA processing/extraction, assessing outside contractors and an attempt to reduce the labour involved in PT evaluation and feedback. Further developments are in progress such as the use of a checklist for the submission of results and the adoption of only three or four software packages for editing. A refinement of the scoring system is also in progress with the revision of virus categories and points with a view to move towards a scoring consistent with that of the virus isolation and ITD PT panels.

### *New algorithm for poliovirus isolation*

A new algorithm for poliovirus isolation was introduced in GPLN laboratories several years ago. The new test allows for the rapid detection of polioviruses by combining the specificity provided by L20B cells and the sensitivity given by RD cells in a series of combined passages that include cross-passages between L20B and RD cell supernatants. The method focuses primarily on the isolation of poliovirus. The main changes involved shortening the period of observation from 7 to 5 days following addition of the stool extract to a cell culture and the removal of the neutralization step used to identify and separate poliovirus serotype mixtures. All L20B positive samples are characterized by molecular assays using real-time PCR which identifies virus-positive cultures as containing non-polio enterovirus and/or poliovirus, which in turn are classified as Sabin, possible WPV or possible VDPV. All programmatically important polioviruses are then sequenced. The PT for the new algorithm for virus isolation is consequently different. The new algorithm is now used in all regions except Europe. It is faster and easier to complete as no neutralization assays are required. However, some laboratories in Europe feared that the new method might affect the quality of enterovirus surveillance, which is an essential

function for some laboratories. There is however no impediment in laboratories further investigating samples using in-house procedures outside their duties as WHO network laboratories. Although there were some requirements for laboratories using older algorithms, such as the need to use both L20B and RD cells, the reality is that there is not a common standardized method in use in the Region. Laboratories in this Region have a broad range of experience and laboratory techniques. Several countries have no acute flaccid paralysis surveillance but rely on alternative surveillance systems. For these reasons, many laboratories do not adhere strictly to the standardized methods for polio diagnosis recommended and supported by WHO. Following the review of data worksheets and information shared by laboratories during the last PT exercise, it was noted that laboratories use many different cell lines. As many as 13 different cell lines were used in all laboratories and as many as 5 different cell lines in a single lab. There was excessive cell culture work done in general with some laboratories performing 2-3 serial passages on the same cell line but many laboratories not performing cross-passages between human cells and L20B cells. There was also a significant number of neutralization experiments performed on many different cell lines, sometimes including L20B-negative samples in polio neutralization tests which increased the risk of contamination and the demand for reagents. Some laboratories used molecular methods including direct PCR detection, RT-PCR and sequencing which should not be used for a virus isolation PT.

A change to the new algorithm was proposed during the Meeting of the Polio Reference Laboratories in the European Region held in Istanbul in 2013. This possibility is currently being evaluated by the WHO Regional Office in consultation with global, regional and national laboratories and national authorities. Although changing to the new procedure would not result in substantial changes in laboratory methodologies, its adoption might have serious implications in terms of changes required for logistics, quality assurance, national policies, etc. It is also likely that with the new algorithm the number of samples that need to be tested/sent for ITD characterization increases. A survey was sent to all laboratories with a number of questions that would help in making a final decision. Inquiries were made on cell culture procedures and quality assurance systems used in the laboratories as well as the possible legal aspects of switching to the new method. Questions were also included requesting the willingness of laboratories to adopt the RT-PCR ITD method, asking them to report any previous experience using real-time PCR for the clinical diagnosis of pathogens. Based on the responses to the survey and the need for rapid polio diagnosis using standardized protocols across the Region, a decision

was made to recommend that all laboratories adopt the new algorithm for poliovirus isolation. A number of laboratories will be selected to perform rRT-PCR ITD tests depending on laboratory conditions and programme needs. A detailed plan for implementation has been drawn which includes an all-laboratories consultation to be held in September 2015. Training workshops for ITD are planned as well as amendments to the laboratory data management system (LDMS) reporting system. All members of the network already received an official communication about the decision. WHO will support the process by organizing training activities, evaluating performance through PT and providing rRT-PCR ITD kits to those laboratories that will be performing the ITD. However, laboratories that would choose to implement ITD will rely on their existing real-time PCR machines and purchase enzyme reagents themselves.

### *Global Action Plan III for poliovirus containment*

An ambitious strategy has been developed for the Endgame of GPEI, which initially requires the withdrawal of OPV2 from tOPV for routine vaccination. Several prerequisites are needed to accomplish this phase including verification of the interruption of circulation of WPV2, implementation of surveillance and response protocols for type 2 poliovirus (including constitution of a stockpile of monovalent OPV2), introduction of at least one dose of inactivated poliovirus vaccine and access to licensed bivalent OPV (bOPV) for routine immunization. It would also be necessary to complete Phase I of containment for type 2 poliovirus, with appropriate handling of residual type 2 materials. The trigger for setting up a date for the withdrawal of type 2 OPV will be determined by the global absence of persistent cVDPV2. Complete withdrawal of OPV and switch to the use of IPV only for routine immunization will occur soon after global eradication is achieved. These changes will surely have an impact on surveillance activities, laboratory testing and containment requirements. Laboratories should be aware of this situation and be prepared for all requirements and deadlines set up for each phase as described in the 3rd Edition of the Global Action Plan for poliovirus containment (GAPIII). As things currently stand, phase I consists of global coordination and readiness for OPV2 withdrawal and should be completed before the end of 2015. Phase II covers the period of global containment of poliovirus type 2 (2016–2018) and phase III will deal with long-term containment of all polioviruses and is expected to take place from 2019 onwards. The process will start with WHO regional directors sending a letter to ministries of health of all Member States requesting them to initiate the process. As an inventory of activities for WPV infectious

and potentially infectious materials was completed in most countries some years ago, revising available reports, documents and checklists might be useful.

It is expected that institutions willing to become essential poliovirus facilities are certified by national regulatory authorities responsible for containment (NRAcs) to meet GAP III requirements for handling and storage of WPV2 (Annex 2) before the end of 2015. For handling and storage of OPV2/Sabin2 materials, facilities should be certified to work in containment (Annex 3) by July 2016. It is important that NRAcs recognize that GAPIII is a performance-based document that allows for different solutions to achieve safety and security while handling poliovirus. All non-essential facilities likely to investigate new VDPV2 isolates should adopt a biorisk management system (Annex 6) before the end of 2015. WHO is offering support to facilities (laboratories and vaccine manufacturers) and auditors: a number of meetings and training courses for GAPIII implementation have been or will be organized to help with this process in all WHO regions. WHO does not have the mandate to certify facilities, but will perform verifications of GAPIII compliance at the request of the regional certification committees, NRAcs or concerned facilities. A number of key questions remain, such as what level of control is expected, what degree of assurance is required to ensure that controls are being effectively maintained and what forms the basis for the scheme. It will also be important to establish who and how the process will be overseen nationally and internationally and who will perform the different activities. Finally, resources would also need to be identified to complete this process. These questions will be addressed in the new publication '*GAPIII Containment Certification Scheme*' that is currently being developed.

## Recommendations

1. Laboratories of the WHO Europe Polio Laboratory Network should maintain high levels of performance in a context of competing public health priorities, budget constraints and changes in clinical diagnostic techniques. 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 polio surveillance.
    b. Regional and national reference laboratories should maintain frequent communication to help monitor performance, facilitate the shipment of PV isolates for ITD

characterization and ensure there is adequate follow-up when performance issues are identified.

2. Maintaining high levels of laboratory quality assurance according to the WHO accreditation process is essential. To achieve this:
    a. Laboratories should use methods that have been standardized and validated by WHO for which PT evaluation is possible.
    b. Laboratories should perform PT procedures following recommended instructions, which include reporting results on time and in the right format.
    c. Global specialized laboratories responsible for PTs and accreditation should provide detailed feedback of results to laboratories emphasizing any weaknesses identified and the possible consequences for the sensitive and accurate detection/identification of polioviruses.
    d. Laboratories should perform cell sensitivity testing regularly according to WHO recommendations and SOPs. Laboratory directors, with support from associated regional reference laboratories, should ensure that cell sensitivity results are properly evaluated and that any necessary corrective actions are promptly taken.
    e. Laboratories should use Master and Working Cell banks that have been prepared from authenticated cell lines received from global specialized or regional laboratories.
    f. The Regional Office will propose a common protocol for testing for the presence of mycoplasma in cell cultures to be used by all laboratories.

3. Laboratories should adopt the new algorithm for poliovirus isolation. To achieve this:
    a. Laboratories should prepare appropriate SOPs, worksheets and reporting forms for the new test following instructions from the Regional Office.
    b. The Regional Office should explain the PT process for the new test including requirements for reporting and the scoring system.
    c. The Regional Office should identify laboratories that will implement the rRT-PCR ITD method and provide support by supplying reagent kits and organizing training workshops.

4. Laboratories should implement the improved rRT-PCR ITD 4.0 method following recommendations by 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 SOPs to implement and validate the new technique as instructed by WHO.
c. Laboratories with low workloads should ensure that regular testing (at least once every three months) is performed to maintain competence. Samples from previous PT panels or Sabin reference strains could be used for this purpose
d. Global specialized and regional laboratories should commit to the adoption of the method for elution of viral RNA form FTA cards recommended by WHO, as using FTA cards will greatly simplify shipping isolates for ITD by national laboratories. Training videos for both adsorption and elution of viral RNA to and from FTA cards are available from CDC.

5. Ensuring a continuous flow of high quality data is essential to assess and follow up laboratory performance and provide essential information to the programme. To achieve this:
    a. Selected laboratories should complete the annual report and accreditation modules of the GPLN Management System and send feedback and suggestions for changes to the Global Laboratory Coordinator.
    b. The interpretation of molecular epidemiological data generated by laboratories should be coordinated between regional/global laboratory coordinators and scientists from global specialized laboratories to ensure that accurate chronological and geographical links are established between PV isolates from different areas, countries or regions as this provides essential information to guide GPEI activities. This is particularly important for the correct classification of VDPV isolates found in the different surveillance activities.
6. Training for biorisk management and GAPIII implementation 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 facilitate the successful establishment of a biorisk management system according to GAPIII.
    b. Surveillance activities should be carried out in facilities implementing appropriate biorisk management systems. RRL senior management 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. Laboratories should be aware of the implications of GAPIII and should follow recommendations for laboratory containment set up in the GAPIII document. WPV2 should be destroyed or contained by end–2015. OPV2/Sabin2 materials should be destroyed or contained by end–July 2016. A procedure for destroying poliovirus will be included in the revised WHO *Polio Laboratory Manual*.

**The WHO Regional Office for Europe**

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

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