

The 22nd Informal Consultation on the Global Polio Laboratory Network

9-10 March 2016, WHO Headquarters, Geneva, Switzerland

- Final report of recommendations -

BACKGROUND INFORMATION

The main commission of the WHO Global Polio Laboratory Network (GPLN) is to ensure that laboratory procedures and work practices are in line with the Polio Eradication Endgame and Strategic Plan's (PEESP, for years 2013-2018) objectives to better inform and orient the Global Polio Eradication Initiative (GPEI). Principal aims of PEESP are (1) to detect and interrupt PV circulation (2) to strengthen immunization systems and support the withdrawal of OPV for immunization (3) to certify PV eradication and achieve PV containment and (4) to plan the use of the vast experience developed by the GPEI as a legacy for other public health programs to control and eliminate infectious diseases.

The Global Polio Eradication Initiative is running directly under the WHO Director General and Dr. Michel Zaffran, the WHO Director of Polio Eradication, reports to WHO DG.

The 22th Informal Consultation of the WHO Global Polio Laboratory Network (GPLN) was held in Geneva, Switzerland, on 9-10 March 2016. The meeting included participants from laboratories of the six WHO geographical regions including WHO Laboratory Coordinators and representatives of the 146 member laboratories of the GPLN. The main discussions focused on laboratory issues of relevance to the Global Polio Eradication Initiative (GPEI) and especially on the current situation of poliovirus transmission, expansion of environmental surveillance, global switch from tOPV to bOPV, diagnostic needs after withdrawal of Sabin 2 from trivalent OPV and laboratory organization and work under GAP III containment.

A. GLOBAL OVERVIEW ON POLIO ERADICATION

Dr. Michel Zaffran, the WHO Director of Polio Eradication, opened the meeting by presenting an overview of progress on the Polio Eradication and Endgame Strategic Plan 2013-2018. He reviewed the current state of the GPEI by highlighting considerable progress achieved in Polio Eradication in the past year. Africa has been free of wild poliovirus since July 2014. The global eradication of wild poliovirus type 2 was certified in September 2015. Likewise, the most recent detection of wild poliovirus type 3

was in November 2012, which leaves type 1 as the only wild poliovirus still circulating anywhere in the world. International spread of wild poliovirus was declared as a Public Health Emergency of International Concern (PHEIC) by DG WHO in May 2014 and temporary Recommendations were made for infected countries. The purpose was to prevent further spread by (1) vaccinating departing travellers (2) emphasizing regional cooperation and cross-border coordination to minimize the risk of international spread. Subsequently, all WPV outbreaks have stopped, leaving endemic circulation in Pakistan and Afghanistan as the only source of wild polio circulation. In November 2015 the recommendation was extended to cover cVDPV-infected countries. The known risks of continued use of tOPV outweigh the risks of OPV2 cessation and thus a synchronized switch from tOPV to bOPV will take place globally from 17 April to 1 May 2016. Readiness of countries for the switch has been confirmed during regional workshops and, if necessary, by providing specific financial support for identified needs. After the switch, GAP III specifies that Sabin2 poliovirus should be contained by August 1, 2016. If a type 2 virus is found in human or environmental sources after this date it would mean a failure or breach of phase 2 containment, failure in OPV2 withdrawal, or circulation of Sabin 2-like virus or vaccine-derived poliovirus. Thus, WHO is recommending that all type 2 polioviruses will have to be notified under the International Health Regulations (IHR).

The Global Polio Laboratory Coordinator gave an overview of the progress, performance and workload of the global polio laboratory network (GPLN). Furthermore, an update of the main activities of the network in 2015 together with selected milestones for 2016 were discussed (for details see below)

B. GLOBAL AND REGIONAL UPDATES ON WPV AND VDPV TRANSMISSIONS

The WHO Global and Regional Polio Laboratory Coordinators (GPLC and RPLC) presented detailed information on the performance of polio laboratories from the different regions, providing data on the detection and molecular epidemiology of wild polioviruses (WPVs) and vaccine-derived polioviruses (VDPVs) isolated from the different surveillance activities.

Only one serotype of wild poliovirus (type 1, WPV) has been detected since November 2012. All outbreaks caused by wild type 1 have stopped, leaving endemic circulation in Pakistan and Afghanistan as the only source of wild polio circulation. Another good sign for the program is that the genetic diversity of wild poliovirus type 1 has declined during the 2015 high season. However, recent outbreaks caused by cVDPVs were reported from Lao People's Democratic Republic (cVDPV1), Guinea (cVDPV2), Myanmar (cVDPV2), Madagascar (cVDPV1), Ukraine (cVDPV1) and Nigeria (cVDPV2).

It was highlighted in several presentations that, in view of the polio endgame, VDPVs have become as relevant for the GPEI as wild polioviruses. We need to optimize the sensitivity of surveillance for VDPVs to allow the quick identification and reporting of these isolates. It is clear that present delays in obtaining the final classification of VDPVs have to be eliminated. The new VDPV guidelines (2015) are useful as they clarify and standardize definitions and roles / responsibilities, particularly at the regional and country level. In addition, all laboratory personnel and programme staff should be aware of the field investigation requirements described in the VDPV Reporting and Classification Guidelines. Since sequencing data is essential for classification, the sequencing laboratory must play a critical role in the

final classification of VDPV isolates. The national programme should encourage field staff to do clinical and field investigations (coordinated by the), and share results with the RPLC. Furthermore, it was emphasized that the reporting of sequencing results should be clear, to avoid misunderstanding; a statement with standard wording should be provided together with the sequence data.

The 146 network laboratories with diverse but well-defined functions have shown their capacity to adapt to changing situations in the GPEI. Altogether in 2015, 192,250 stool specimens were studied and more than 8500 poliovirus isolates characterized. Furthermore, 36 laboratories tested 7262 sewage specimens for poliovirus. They were characterized by the timely and accurate detection of WPV and VDPV, even in the absence of AFP cases. In 2015, all laboratories in the network used the new cell culture algorithm for poliovirus isolation. The laboratory performance during 2015 was good. However, if we want to sustain the excellent performance levels of previous years, efforts need to be more focused. The National polio Laboratories in Syria, Iraq and Maiduguri were specifically thanked since they have been able to continue working under dangerous security situations.

To facilitate global polio data collection and gathering, the GPLNMS-reporting system was developed for the network. For 2015, as of 10 March 2016, only 50 % of laboratories completed annual reports through GPLNMS. Reporting through GPLNMS will be mandatory in 2016.

The Global Polio Laboratory Coordinator also emphasized that a major threat to GPLN performance is the high workload faced by Laboratory coordinators, specifically when the GPLN and the Programme are entering a turbulence zone where expectations will be high.

GAP III containment

The known risk of continued use of Sabin 2 in tOPV is not acceptable at this phase of eradication as wild poliovirus type 2 circulation has not been detected for more than 15 years. The global synchronized switch from tOPV to bOPV will take place from 17 April to 1 May 2016. After the switch, GAP III specifies that type 2 polio viruses including Sabin2 have to be contained by August 1, 2016. This means that viruses have to be either destroyed or transferred to an essential poliovirus facility.

Likewise, all polio potentially infectious materials will be under GAPIII containment requirements, which mean they have to be destroyed or sent to an essential facility. It was emphasized that there is no test assay by which potentially infectious materials can be shown to be negative and stored further without containment. Since the current definition of "potentially infectious materials" includes all stool samples and respiratory materials from periods when and where the country/region was using tOPV, the issue is much wider than the GPLN. It has implications for all laboratories handling respiratory or gastrointestinal pathogens (for example rotavirus, influenza virus and different bacteria).

In his presentation, the Global Polio Laboratory Coordinator demonstrated what kind of changes the GPLN has to face in handling poliovirus diagnostics post-OPV2 withdrawal. The old structure of GPLN network laboratories might need to be changed significantly. The GPLN has already gone through several changes, mainly due to the (1) changes in diagnostic methods (2) capacity building to align polio diagnosis to programme needs (3) adjustments to changes in shipping regulations and Courier's requirements. However, now with the need to comply with GAPIII containment requirements, we face a completely changed landscape. This might mean the need to review the terms of reference of many

polio reference laboratories (new ToRs). Implementation of GAPIII requirements is in emergency mode and it means containment for type 2 poliovirus (from July 2016 onwards). The most important current question for national polio laboratories is how to implement the necessary changes without hampering diagnostic capacity.

The majority of polio laboratories will work under GAPIII Annex 6 – which means they can assay specimens for poliovirus by virus cultivation, i.e. work as poliovirus isolation (PI) laboratories, but they should immediately send all L20B positive cell cultures to a PII (poliovirus isolation and ITD) or a PIIS (poliovirus isolation, ITD and sequencing) laboratory for ITD analysis. PI laboratories will only store materials which potentially contain poliovirus for short periods of time, i.e. until ITD and sequencing results are ready. Within 72 hours of the final sequencing results being available, the specimens together with all virus-derived materials will be either appropriately destroyed or sent to an essential polio facility. Likewise, the PII laboratory will send all type 2 polioviruses as well as any other relevant type 1 and 3 poliovirus identified in ITD assays to a PIIS laboratory for sequencing. Again, within 72 hours of the final sequencing results being available, the specimens together with all virus-derived materials will be either appropriately destroyed or sent to an essential polio facility. Essential facilities are the only ones that can store materials under containment for longer periods of time.

The Regional Polio Laboratory Coordinators presented detailed information on the progress of type 2 containment in their regions. They concluded that current timelines required to complete containment are very difficult to meet. Those for endemic countries are the most difficult. Further challenges have been identified in non-polio laboratories. Many do not understand the implications of GAP III for polio containment on their laboratory work. More communication is required and especially between WHO Polio Containment team and the different infectious diseases control or elimination programs.

According to the experiences of the Regional Polio Laboratory Coordinators, who are also responsible for containment, (1) strong political commitment towards laboratory containment of WPV is a must, (2) the laboratory survey and inventory process has effectively identified laboratories with WPV/VDPV materials, and (3) the documentation process serves a critical role for compiling information that will be important for subsequent phases of certification. They also reported that, in spite of their efforts, they have faced the following problems: (1) lack of support from MoH, (2) lack of legislation for laboratory registration, (3) no list of laboratories available at national level, (4) engaging with multisectoral laboratories is not obvious, and finally (5) limited capacity of containment coordinators/advisors for data management: entry, cleaning, analysis.

Expansion of Environmental Surveillance

The Global Polio Laboratory Coordinator reported on the good progress made in the expansion of environmental polio surveillance. The programme follows the Environmental Expansion Plan adopted by WHO after consultation with the different stakeholders.. The objectives for expansion are (1) to help identify any residual transmission in endemic and re-infected areas (2) to provide early indication of new poliovirus importation, (3) to rapidly detect any new emergence of VDPV and document the elimination of Sabin viruses following the withdrawal of OPV2 and eventual cessation of all OPV. It was also highlighted that ES will be a key strategy to monitor the effectiveness of containment in facilities.

The laboratories for expansion have been selected in three key regions (AFR, EMR, SEAR) and establishment of activities and training are well in progress. In 2015, the capacity building and laboratory strengthening were focused on selected high priority countries in Africa and Asia. As of December 2015, the expansion plan is on track, excepted for Iraq due to security reasons. The target (end of 1st quarter of 2016) will probably be missed in Yemen and Somalia for same reasons. . In 2015 training workshops were organized in South-Africa, India and China. In several countries, training was followed by a specific on-site support visit to the laboratory before implementation of environmental surveillance of polioviruses was initiated. Subsequently, environmental surveillance of poliovirus in sewage was established in following Cameroon, Niger, Chad, Burkina Faso, and Madagascar in Africa; Bangladesh, Myanmar and Indonesia in SEAR and Mexico and Haiti in PAHO have started in the first quarter of 2016. In EMR, the expansion process in 2015 has focused on new sampling sites in Pakistan and Afghanistan.

According to the WHO Global and Regional Laboratory Coordinators, the major constraints in the ES expansion plan have been (i) the lengthy administrative process to procure ES equipment and supplies to new laboratories and (ii) buy-in and delayed response from countries to establish ES due to security issues. Furthermore, delays, hurdles and/or problems were caused by lengthy implementation processes, aligning GPEI partner's visions and poor involvement of surveillance personnel.

Participants to the meeting have reiterated the notion that Environmental surveillance expansion will require considerable resource investment and should not jeopardize GPLN capacities to support AFP surveillance. STOP

To help implementation of environmental polio surveillance, the protocol of the WHO recommended method for sewage concentration, 2-phase separation, was harmonized, and the document "Guidelines for Environmental Surveillance of Polio" prepared (available at the WHO website). Both documents will be updated before June 2016. Likewise, short term studies to optimize the 2-phase separation protocol and reduce the need for ITD reagents are in progress (see diagnostic assays). For expansion of environmental polio surveillance in sewage, highly sensitive diagnostic assays are needed. Detection of minor components of complex virus mixtures typically present in sewage is challenging and discrimination by sequencing even more difficult. Without highly sensitive rule-in rRT-PCR assays, detection of homotypic mixtures is impossible.

The new suggested proposal (not approved yet) on further expansion of environmental polio surveillance (ES-expansion) to additional 200 sampling sites in 20 new countries within the coming year was presented by the global laboratory coordinator. When considering further expansion it is important to remember that (1) implementation of ES is not easy (2) samples are difficult to process and characterize and (3) limitations of the method; positive results are useful, but negative results don't mean anything. Furthermore, security and access issues must be addressed in setting up new labs.

During subsequent discussions it was highlighted that expansion of environmental polio surveillance in sewage will require considerable resource investment and should not jeopardize GPLN capacities to support AFP surveillance. One technician can handle 8 sewage samples per week. Since several of the existing polio laboratories are overloaded new personnel should be hired but also new space /

laboratories found. Furthermore, Regional Polio Laboratory Coordinators together with scientists from GSLs pointed out that (1) establishment of the method in countries with civil wars is challenging (2) 6-9 months minimum is required to establish the method for laboratories who already work with polio, whereas much more time is required for those who are not already working on poliovirus (3) in countries and laboratories that do not have capacity and capability to do the work, shipment of specimens to another laboratory could be an option, (4) The capacity of RPLC and GPLC exceeds the demands of lab networks; resources need to be identified to meet these needs. It was also highlighted that the risk assessment of environmental polio surveillance should be revisited since the new expansion proposal will be primarily targeted to VDPVs while the focus of the previous one was on wild polio virus. Another concern came up in the discussion on the length of environmental polio surveillance. There was a clear agreement that it should be temporary, covering the time period relevant to the question and certainly not to be continued forever. The quality of environmental polio surveillance in sewage was highlighted in several discussions. It is not only the number of sites and samples that matter but also the quality of work. Therefore, an accreditation checklist for environmental polio surveillance should be completed and pilot tested as soon as possible. Furthermore, newly established laboratories should be followed by frequent on-site visits. To facilitate management and implementation of the ES-expansion, an environmental polio surveillance implementation group will be set up with the following terms of reference: (1) Standardized monitoring indicators to track effectiveness of ES (2) Guidance and recommendations on management, analysis, and reporting of ES data (3) Review human resources and other resource requirements for full integration of ES and AFP surveillance (4) Ensure sustainability of the integrated surveillance system beyond 2016 .

Diagnostic assays

When global eradication is achieved, any live poliovirus will be a potential emergency. Rapid identification of a “hot case” is a must and poliovirus-containing specimens will have to be rapidly flagged for sequencing. Furthermore, primary testing should be as close as possible to the AFP case. All this means that there will be increasing demand for improved methods with better sensitivity, specificity and more rapid turnaround time. Likewise, as eradication progresses, the inactivation of potentially infectious clinical materials and virus isolates will become a prerequisite for the work. The scientist from CDC presented their data on virus inactivation. They analyzed different temperatures and incubation times to determine the best conditions for virus inactivation. Under GAPIII containment, FTA cards will become a critical mechanism to send inactivated polio isolates from one laboratory to another for molecular testing.

The scientist from CDC presented that development and implementation of new modified ITD assays are progressing. Recently, ITD testing sensitivity was increased 10 to 100-fold with the introduction of ITD4.0. However, it was realized soon that ITD4.0 assays have problems in reactivity with some wild type 1 and 3 polioviruses. Now the kit has been further improved by providing two new redesigned reaction assays together with more stringent reaction conditions. After completing pilot testing, ITD4.1 kits will be sent to laboratories. The new assays are direct replacements for Duplex WPV1 and AFR

WPV3 of ITD 4.0. Thus the distribution to the laboratories can be done so that ITD4.0 kits will be sent together with an addendum ITD4.1 kit until all ITD4.0 kits are exhausted. Laboratories will be asked to replace the corresponding two ITD4.0 tubes with the two new ITD4.1 tubes.

The next change in ITD kits will take place around July, 2016 when the version ITD5.0 will become ready for implementation. The kit contains a newly designed assay for type 2 polioviruses. This assay is designed to detect Sabin 2 viruses, VDPV2, and wild type reference strains such as MEF-1. At this time, there is no plan to include a rule-in VDPV2 assay because all type 2 polioviruses will be sequenced after August 1, 2016.

As expansion of environmental polio surveillance in sewage progresses, a huge increase will be seen in the demand for ITD reagents. Short term research needed for the optimization of 2-phase separation (the WHO recommended method for sewage concentration) was discussed in the harmonization workshop in 2015 and the following four topics were identified (1) fate of pellet (2) chloroform treatment needed to clear bacteria contamination (3) number and size of cell culture flasks needed for virus isolation (4) number of ITD reactions/ specimen. The aims of these studies are to simplify and speed up the protocol and reduce the need for ITD reagents. The results obtained to date were presented by the participating GPLN laboratories and discussed during the meeting. However, the number of specimens studied was still low and more results are needed for a final conclusion.

The scientist from CDC reported promising results on direct detection of polioviruses in stools. Pilot study on stool samples is being planned for Pakistan. In the detection assay, enrichment of viruses was based on magnetic beads coated either by poliovirus receptor or virus specific antibodies. However, the binding capacity of magnetic beads varied from one lot to another and thus their quality should be checked and standardized before starting analysis. Direct detection of polioviruses from sewage have also been studied in CDC, Israel and NIID Japan. Although some progress has been reported, and specific strains could be detected from sewage without virus isolation, the major remaining challenge is detection of programmatically important minor components of complex virus mixtures present in sewage.

C. PERFORMANCE IN PROFICIENCY TEST PANELS

As in previous years, careful reviews of the GPLN's quality assurance program were presented by the scientists from GSLs with information and conclusions on the results from annual proficiency testing activities used to assess the performance in the different laboratory techniques. Quality assurance is one of the strengths of the GPLN. Performance in 2015 was still good even though they are not as excellent level as it was in 2014. Indeed only 79 virus isolation laboratories out of 98 who have submitted the results got the maximum score. Four laboratories failed (one from EURO, one from AFRO and 2 from SEARO). Implementation of corrective actions is ongoing for the 4 laboratories. Altogether, there are 88 ITD laboratories and 19 sequencing laboratories were accredited. VP1 sequencing has been included in the QA program for the past 4 years and improvements continue to be made. The four samples in the next Sequencing PT will consist of lyophilized noninfectious RNA transcripts as usual but in addition to the 4 samples, there will be a pilot testing for FTA card

processing, consisting of a single FTA card that will be subjected to RNA processing and VP1 sequencing.

D. LEGACY AND ROLE OF POLIO LABORATORIES AFTER ERADICATION

The last session of the meeting was dedicated to group works where participants were divided into small groups (one group / region) to discuss following topics.

(1) the regional specimens' referral scheme after 31st July 2016 (deadline for containment of OPV2 and Sabin2).

(2) regional network orientation post-eradication

The following aspects were raised:

(1) the GPLN has practical knowledge of how to create and maintain functional global public health networks and can contribute to the long-term goal of having strong public health laboratories in every country,

(2) Environmental surveillance could be used to expand the search for other viruses/infectious agents to link with other programs.

(3) Implementation of Biorisk Management Systems in GPLN laboratories is a legacy that can be applied to other infectious agents.

Informal Consultation Recommendations:

1. All lab personnel and Programme staff should be aware of the field investigation requirements in the VDPV Reporting and Classification Guidelines. The sequencing lab must play a critical role in the classification of VDPV, including encouraging field staff to do clinical and field investigations (coordinated by EPI), with the process coordinated by the RPLC.
2. Take the recommendation from SWG: VDPV classification should be a coordinated decision making process.
3. The GPLN should develop standardized reporting text for email messages accompanying sequencing reports.
4. The implied definition of cVDPV that has replicated extensively (1.5% VP1 nt. difference) in the VDPV Guidelines should be removed or clarified.
5. An Environmental Surveillance Implementation Group should be convened before the next SWG meeting.
6. Document the challenges and resource needs for rapid ES expansion as soon as possible, coordinated by GPLC and HQ Surveillance.
7. An ES Accreditation Checklist should be piloted and completed by the end of 2016.
8. Rollout of new ITD versions:
 - a. Most labs should continue to use ITD4.0 kits in their possession and to test the next ITD PT panel using ITD4.0. Pilot labs should use 4.0 and 4.1 in parallel.

- b. After completion of the pilot, ITD4.0 kits will be sent together with an addendum ITD4.1 kit until ITD4.0 kits are exhausted. Labs will be asked to replace the corresponding two ITD4.0 tubes with the two new ITD4.1 tubes.
 - c. We anticipate that ITD5.0 will replace ITD4.0 and 4.1 after the current inventory of ITD4.0 kits are exhausted and recommend that CDC develop a plan for ITD 5.0 piloting and implementation.
 - d. Recommend that CDC develop detailed protocols, worksheets, reporting forms, etc. that can be distributed with the ITD panels.
9. Recommend that WHO develop QA (accreditation checklists) and assessment tools directed toward GAPIII.
10. Recommend that RPLC align specimen referral patterns of biological materials with GAPIII after OPV2 withdrawal. Include contingency plans; provide feedback on proposed adaptation of testing algorithm to GAPIII.
11. Recommend that the RPLCs work with the GPLC to identify remaining obstacles (contingencies) to implementation of universal FTA Card referral for both the shipping and receiving labs.
12. GSLs should, in consultation with the GPLC and RPLC, identify their short term and long term needs, particularly human resources, in order to support the GPLN in the most critical activities, with the goal of updating the ToRs and work plans for each GSL, by the end of April 2016.
13. Participants to provide feedback on the proposed pathway diagrams for PI, PII, PIIS labs by March 18, 2016.
14. The GPLN should develop guidance for handling PV diagnostics in non-essential facilities for polio containment, by end of June 2016.