



**World Health
Organization**

REGIONAL OFFICE FOR **Europe**

**Meeting of Reference Laboratories
Polio Laboratory Network of the WHO European
Region**

**Istanbul, Turkey
30 September–2 October 2013**

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Summary

A meeting of the national, regional and global specialized polio reference laboratories in the European Region of the WHO Global Polio Laboratory Network (GPLN) was held in Istanbul, Turkey. The meeting included representatives from 30 member laboratories, the United States Centers for Disease Control and Prevention (CDC), the European Centre for Disease Prevention and Control (ECDC), the European Polio Regional Certification Committee and WHO regional and headquarters offices.

Representatives from WHO opened the meeting by reviewing the current state of the global polio eradication initiative (GPEI) giving details of planned endgame strategies and results from risk assessments for wild poliovirus (PV) transmission in the European Region. An overview of the performance, activities and challenges faced by GPLN laboratories was presented by the global and regional coordinators. The performance of the Polio Laboratory Network in the European Region was reviewed extensively by laboratory directors from regional and global specialized laboratories with associated national polio 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 PVs and vaccine-derived polioviruses (VDPVs) continue, as international shipment of materials and reagents such as virus panels, cells, clinical samples, etc., is getting increasingly difficult and expensive due to strict regulations in most countries.

A CDC representative presented an update on the strategic plan for environmental (ENV) surveillance of PVs and discussed future changes in polio diagnostic protocols, with a 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 GPEI endgame strategic plan. Efforts to develop improved laboratory methods and standardized quality assurance procedures continue to be a priority of the GPLN.

The need to maintain high performance in a context of changing public health priorities, budget constraints and a shift to the use of molecular tests for the laboratory diagnosis of infectious diseases in most countries was considered a priority by all participants. Participants also discussed the possible adoption of the new algorithm for poliovirus isolation, which allows results to be obtained more rapidly and is used in all other WHO regions. This possibility is currently under consultation among laboratory directors.

Discussions

1. Progress towards global polio eradication – Endgame

We are closer than ever to global polio eradication. The number of cases of paralytic poliomyelitis is low; there have been no polio cases due to wild PV type since 3 November 2012; and India, where large epidemics were frequent until recently, has not seen a polio case due to wild type virus since February 2011. However, several concerns and challenges remain: three countries have still not been able to interrupt circulation of indigenous wild PV; there is a large outbreak of wild type 1 PV in Kenya, Ethiopia, Somalia and Sudan; and small outbreaks of type 2 circulating VDPVs (cVDPVs) continue to occur in parts of central Africa, Afghanistan and Pakistan. Furthermore, wild type 1 PV has been found in ENV samples from Egypt and Israel indicating widespread virus circulation (particularly in Israel) in the absence of known paralytic cases. Risk assessments carried out in Europe based on surveillance indicators, population immunity and other risk factors related to the quality of public health services indicate that, although many countries in Europe present low risk of PV transmission, there is still a significant number of countries with medium or high risk for wild PV transmission from importation and/or establishment of VDPV circulation. The main reason for this appears to be the fact that there are insufficient surveillance activities in some of those countries; and in some cases deficient public health services can cause interruption of immunization activities and poor public health assistance in general.

An ambitious strategy has been developed for the endgame of GPEI which includes the sequential withdrawal of type 2 and type 3 live-attenuated oral polio vaccine (OPV) from the trivalent OPV preparation for routine vaccination. Several requisites are required to undergo each phase, which include certification of the interruption of circulation of wild PVs and cVDPVs of the corresponding serotype and the availability of inactivated polio vaccine (IPV) for global use. Complete withdrawal of OPV and the 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 that would need to be anticipated and prepared for.

2. Performance of the Global and European Polio Laboratory Network

Performance of the Polio Laboratory Network in the European Region remains high. All participating laboratories in Europe are fully accredited. All laboratories are now reporting through the Laboratory Data Management System (LDMS) although some concerns remain on the consistency of reporting, as reports vary in quantity and frequency. Although the quality of reporting measured as the percentage of laboratories reporting with more than 80% completeness has improved slightly in 2013 with respect to 2012 (56% versus 40%), it is still far from optimal. Many laboratories still do not understand the concept of weekly “zero” reporting. There are also added concerns in that some countries are reporting very few or no PV and/or non-polio enterovirus (NPEV) isolates. Challenges to improve timeliness of detection of wild PVs and VDPVs continue, particularly those concerning the shipment of stool samples and PV isolates to national laboratories or regional reference laboratories.

During 2012, 96% of isolations of PVs and 84% of ITD results in the European laboratories were obtained within the required timelines. A total of 8038 specimens from all surveillance activities were processed. Of those, 4283 were faecal specimens from cases of acute flaccid paralysis (AFP). No wild PV isolations were reported during this year. Two type 1 VDPVs, 3 type 2 VDPVs and 2 type 3 VDPVs were isolated from various sources in Israel, Turkey and the United Kingdom in 2012. A type 2 VDPV isolate with 4.4% VP1 sequence divergence from Sabin 2 was identified in Germany from a 2 year-old girl from Saudi Arabia who was hospitalized. One type 1 aVDPV and one type 2 aVDPV were found in sewage samples from Tampere (Finland) with 13.8% and 15.39% VP1 sequence divergence from Sabin 1 and 2, respectively. A network campaign to ensure laboratories undergo biosafety training and arrange safe transport of samples containing infectious materials continued during 2012-2013.

3. Supplementary surveillance

The European Region of the GPLN prioritizes the isolation and characterization of PVs from faecal specimens from AFP cases supplemented by other surveillance techniques such as ENV and enterovirus (EV) surveillance in its support of the GPEI. ENV and EV surveillance are essential because AFP surveillance is not universally used throughout the Region. More countries are introducing these supplementary surveillance activities into their national programmes.

About 40 Member States report the use of EV surveillance as the only mechanism of polio detection or in conjunction with either AFP or ENV surveillance. Guidelines covering different aspects related to EV surveillance, such as recommended procedures for specimen transport, EV detection and characterization and documentation and reporting of results, are under preparation by the Regional Office. EV was initially adopted as a supplementary activity as it was thought to be a good option to keep the possibility of isolating PV from stool samples in the absence of samples from AFP surveillance. However, there has been a shift in most countries to the use of molecular methods for direct detection of EVs and often the samples used for diagnosis, mostly cerebral spinal fluid, are not suitable for PV detection either by PCR or virus isolation in cell culture. In this context, ENV surveillance for PV is very relevant for the Region and needs to be maintained and expanded in the immediate future in order to supplement AFP surveillance or to substitute for it where AFP is not implemented. There are numerous examples around the world, several in the European region, showing that ENV surveillance is effective and sensitive in detecting circulating wild PV and cVDPV as well as VDPV strains presumably shed by one or few individuals even in the absence of known paralytic cases due to polio.

About 20 Member States report ENV surveillance activities at the moment. However, standardization and establishment of a quality assurance programme for ENV surveillance is complex, as virus isolation rates might vary significantly between countries/regions. Many variables can affect isolation rates from ENV samples such as differences in sewage infrastructure, population density, national immunization policies, climatic conditions, migration movements, etc. Methods for sample collection and virus isolation algorithms might also vary between laboratories, and virus isolations are often not performed in WHO-accredited laboratories, which makes evaluation of performance and tracing of results more complicated. Efforts are on-going to develop updated “WHO Guidelines for ENV Surveillance of PV circulation” and detailed standard laboratory procedures to improve the efficiency of analysis of sewage samples. These control systems should help in identifying quality indicators to assess the extent and effectiveness of these supplementary surveillance activities.

4. Laboratory quality assurance

4.1. Proficiency testing (PT) programme

The annual PT and assessment of laboratories continue to be critical for the quality assurance of the performance in polio laboratories. Six different PT panels are in use for evaluating (i) accuracy of virus isolation; ITD by (ii) ELISA, (iii) probe hybridization, (iv) traditional PCR, (v) real-time PCR (rRT-PCR), and, (vi) rRT-PCR for VDPV screening (vii) sequencing PV isolates. The PT programme is coordinated by WHO in collaboration with the GSLs in the United States and the Netherlands. All but one laboratory in the European Region scored 100% in the virus isolation PT for 2012. So far, the majority of laboratories have successfully passed the virus isolation PT in 2013. Irrespective of the final results, 5 laboratories submitted the PT results late. PT analysis should mimic reality as much as possible so delays will not be accepted and, particularly in laboratories with few or no PV isolations, the PT exercise can contribute to the preparedness for outbreak response. All laboratories that attempted the two rRT-PCR panels for ITD and VDPV screening and/or the sequencing PT in 2012 attained passing scores of 100%.

All 48 laboratories were assessed during 2012 and customized recommendations were made for each one of them.

4.2. Cell culture techniques

The quality assurance of all aspects related to the work in GPLN laboratories is critical to guarantee optimal conditions for the isolation of PV and NPEV from stool and ENV samples. The requirement to regularly test the cell sensitivity for PV infection was introduced in the GPLN several years ago and evaluation of results sent by laboratories has proven to be a

very useful tool to monitor laboratory performance and to detect laboratories that were missing virus isolation in samples that were later shown to contain PVs or NPEVs. However, this test is still not used to its full potential in many network laboratories. The concept of cell sensitivity testing appears not to be well understood by most laboratories despite involving one of the most basic techniques in virology, i.e., determining the virus titre. Following assessments and site visits, 18 laboratories in the Region were found to have issues related to cell culture procedures, 4 of them with recurring problems. Common signs of actual problems with cell sensitivity for PV infection and technique 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. It might be necessary to update the accreditation checklist to include evidence for the correct interpretation of results and any action taken following any failed tests. Efforts are on-going to update the chapter describing cell sensitivity testing as part of a general revision of the WHO Polio Manual to be undertaken by scientists from GSLs.

NIBSC has also developed a standard operating procedure (SOP) for cell authentication which has been shared with GSLs. The test permits the detection of mitochondrial DNA corresponding to 1 human cell in a background of 10^5 – 10^6 mouse cells. This makes it possible to detect the contamination of L20B cells with human cells, which can lead to the isolation of an unusually high proportion of NPEVs apparently growing in L20B cells and the unnecessary use of resources for ITD characterization.

5. Implementation of new algorithm for PV isolation

Laboratories in this Region have a broad range of experience and laboratory techniques. Several countries have no AFP surveillance but rely on alternative surveillance systems. Many laboratories have integrated PV diagnosis in EV diagnostic services or in research activities. For these reasons, many laboratories do not adhere strictly to the standardized methods for polio diagnosis recommended and supported by WHO.

A new algorithm for PV isolation was developed several years ago. The new algorithm modified some steps in the virus isolation procedure, which resulted in a significant limitation of the isolation time period required to classify samples as positive or negative for cytopathic effect (CPE) in L20B cells. The main changes essentially 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 PV serotype mixtures. In the new method, all samples that show a positive CPE in L20B cells, regardless of whether they contain a single PV isolate, homotypic and/or heterotypic PV mixtures, are sent to ITD laboratories for characterization. As the demand for quick diagnostic results

increases, the possibility of switching to a new algorithm in European laboratories was discussed. 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. Laboratories have been asked to give feedback through a survey. A decision will be made following assessment but all laboratories would need to agree before the change would take effect.

6. Development and evaluation of new diagnostic methods and reagents

Regional Reference Laboratories and particularly Global Specialized Laboratories (GSLs) continue to contribute to the validation, implementation and improvement of methods used by the GPLN. This is particularly relevant in the context of planned changes for the post-eradication and post-OPV eras, which will include a switch from tOPV to bOPV to mOPV, the introduction of global IPV use and, eventually, the total interruption of OPV immunization. Laboratory algorithms for virus isolation and ITD will need to adapt to ensure that the detection of programmatically relevant PVs is streamlined and prioritized during the different phases of the GPEI endgame. Scientists from CDC are continuously updating protocols for ITD rRT-PCR to help increase the sensitivity and specificity for detection of wild PVs and VDPVs. Future developments in ITD rRT-PCR testing will include a quadruplex assay to detect Sabin 1, 2 and 3 + EV in the same reaction. Primers and probes are also being developed and tested for the detection of wild PVs currently circulating in Africa and the WHO Eastern Mediterranean Region.

Only stool samples and to a lesser extent nasopharyngeal washes are suitable for polio diagnostics. Isolation and characterization of infectious PV in tissue culture remains the most sensitive method for the detection of PV. The development of efficient methods for the direct detection of virus RNA in stool samples is still elusive, which means there are still no accredited rapid molecular assays for the direct detection of PV by PCR as there are for other infectious agents such as influenza or HIV. A variety of PCR methods for EV/PV detection are used in different laboratories but none of them have been proven to be 100% sensitive (as compared to cell culture virus isolation) as is required. It is for this reason that no such method is recommended for use by GPLN laboratories at present. Further development, validation and standardization are required before such a direct PCR method will be available for global use.

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 that adequate logistics, human and financial resources are available for polio surveillance.
 - b. Laboratories should use methods that have been standardized and validated by WHO, for which proficiency testing (PT) evaluation is possible.
 - c. Laboratories should perform PT procedures following recommended instructions, which include reporting results on time and in the right format.
 - d. 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. Laboratories of the WHO Europe Polio Laboratory Network should increase the quality of reporting from surveillance activities using LDMS. To achieve this:
 - a. Laboratories should report results from all AFP, EV and ENV surveillance activities.
 - b. All required fields in the database should be filled so reports for each sample are complete.
 - c. All laboratories should report at least once a week even if no samples were processed during that period (zero reporting).
 - d. The Regional Office can assist if problems using LDMS are encountered by laboratories.

3. There is a need to improve standardization procedures and data analysis for supplementary surveillance activities conducted in the European Region as these are the only means for PV detection in many countries. To achieve this:
 - a. The Regional Office should provide laboratories with revised guidelines for ENV surveillance and new guidelines for EV surveillance.
 - b. A critical review by the participating laboratories in the Member States on the quality of their supplementary surveillance activities, particularly EV surveillance, is strongly recommended.

- c. The Regional Office and reference laboratories should work together to increase the quality of data from supplementary surveillance activities making full use of LDMS for this purpose.
4. Improving the quality of laboratory work with cell cultures should be an immediate priority. To achieve this:
 - a. Laboratories should review their training procedures and SOPs to ensure that they are fit for purpose and follow WHO recommendations.
 - b. 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.
 - c. Results from cell sensitivity assays should be critically evaluated during the accreditation process with clear conclusions and recommendations if needed.
 - d. The possible need for training laboratory staff on cell culture techniques should be evaluated by the Regional Office in consultation with laboratory directors from reference laboratories.
5. Training for biorisk management and promoting biosafety practices in all laboratories should be a high priority for the WHO Regional Office. Laboratory containment activities should continue as required by WHO guidelines and priorities established by the GPEI endgame strategies.