



**Meeting of the Regional Reference
Laboratories (RRLs), WHO EURO Polio
Laboratory Network
Tel Aviv, Israel
3-4 May 2011**

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SUMMARY OF DISCUSSIONS AND RECOMMENDATIONS

A meeting of the Regional Reference Laboratories (RRLs) in the European Region of the WHO Global Polio Laboratory Network (GPLN) was held in Tel Aviv, Israel with representatives from 7 member laboratories of the European Region of the GPLN and WHO laboratory scientists with responsibility for coordinating the network's activities. The European Region of the GPLN prioritizes the isolation and characterization of polioviruses from faecal specimens from cases of Acute Flaccid Paralysis (AFP), supplemented by other surveillance approaches (e.g. testing of sewage waters or enterovirus (EV) surveillance) in its support of the Global Polio Eradication Initiative. Laboratories in this Region have a broad range of experience and laboratory techniques. Environmental and enterovirus surveillance are essential because AFP surveillance is not universally used throughout the Region. In total, 3786 faecal specimens from AFP cases, and approximately 24435 environmental and 111180 specimens from enterovirus surveillance were processed in the European region during the period January – December 2009.

During the meeting, Lab Directors provided updates on their activities over the past 2 years. The Lab Coordinator provided an overview of GPLN laboratories and activities in the Region. The new organizational structure for the WHO European office was presented; the polio program is a part of the Division of Communicable Diseases, Health Security and the Environment in the Unit of Vaccine Preventable Diseases, Immunization, and Influenza. The new online reporting system (Laboratory Data Management System (LDMS)) was demonstrated, and implementation of the system was discussed. The molecular epidemiology of wild poliovirus in this previously polio-free Region was discussed, as well as Vaccine-Derived Polioviruses (VDPVs), aspects of the network's laboratory Biosafety program (videos), and enterovirus and environmental surveillance for the presence of PVs. Recommendations to improve the timeliness of detection of wild type polioviruses (WPVs) and VDPVs were made.

SUMMARY OF DISCUSSIONS

Detection of Wild Polio Viruses

The characteristics of WPVs found during the outbreak of wild type 1 poliovirus in the Region were summarized at the meeting, according to genotype, geographical location and transmission links, based on analysis of the complete nucleotide sequence of the VP1 region of the viral genome. The network detected WPV1s in four countries in the European region. Importation of WPV1 from the Uttar Pradesh region of India into Tajikistan occurred, with cases detected in April 2010. Based on the genetic data, the importation likely occurred in late 2009 or early 2010. WPV1 was subsequently detected in the Russian Federation (15 confirmed cases), Turkmenistan (3 confirmed cases), and Kazakhstan (one case). At least 6 importations from

Tajikistan into the Russian Federation occurred. Limited spread occurred to close contacts of cases in Russia. Epidemiologic investigations of some WPV1 cases found in the Russian Federation suggested links to Uzbekistan, although no WPV has been confirmed there. Turkmenistan cases were located near the border with Uzbekistan. The non-structural region of the poliovirus genome of several isolates has been sequenced. The immunization response was successful in curtailing spread of the virus. The RRL in Moscow measured neutralizing antibody to poliovirus in AFP patients and healthy subjects in Tajikistan in order to learn more about the susceptible population in this area. They found that a large proportion of sera from children born after year 2003 have no neutralizing antibody to type 2 and 3, indicating a gap in the immunity to poliovirus in this cohort.

Detection of Vaccine Derived Polio Viruses (VDPVs)

In September 2010, the GPLN adopted a new definition for type 2 VDPVs, switching from the definition as >9 nt. differences from Sabin 2 in the complete VP1 region to > 5 nt changes in the complete VP1 coding region. The definitions for types 1 and 3 VDPVs have not changed. The upper limit of >15% for all three serotypes was removed and an isolate with >15% is still a VDPV if it can be phylogenetically linked to other VDPV isolates with <15% divergence.

VDPVs have been isolated from various sources in the European region. A VDPV2, unlinked to outbreaks and in AFP case with no confirmed immunodeficiency, was reported from Turkey. The virus was recovered from a healthy child in a stool study being conducted in order to boost the number of specimens processed in this laboratory. The VP1 region had 14 nucleotide differences from Sabin 1 and was classified as Sabin-like in the ELISA test.

VDPVs were also reported from non-AFP specimen sources. The VDPVs from environmental specimens are likely from one or a few long-term excretors with B cell immunodeficiency. Genetically linked VDPVs of all 3 serotypes have been found periodically in the sewage in Tampere, Finland since December 2008, despite the fact that IPV has been in use in Finland since 1960 (the only exception was the use of OPV in response to a WPV3 outbreak in 1984). More than 70 VDPV strains have been isolated over several years, and the VDPVs have probably been evolving for more than 12 years based on the level of sequence divergence from Sabin strains. A stool survey of individuals identified with primary B cell immunodeficiency is planned to commence in 2011. VDPV2s have also been identified in sewage in Tallinn, Estonia in 2010. The genetic divergence of these VDPVs from Sabin 2 in the capsid region is > 13%, corresponding to more than 13 years of evolution based on the poliovirus molecular clock.

Periodic detection of VDPV2 in sewage has also occurred in Israel during the past 12 years. All strains are Sabin-Sabin recombinants, and there were two genetically distinct groups of VDPV2 viruses detected in 2010. Attempts to identify the excreting individuals have been unsuccessful to date. A study involving the spiking of Sabin virus into the sewage system is in progress in Israel in order to determine the limits of detection of the environmental sampling process.

Prolonged VDPV detections are of concern because of their potential to circulate. Another concern is that frequent reporting of VPDVs to public health authorities in the absence of an identified source, despite appropriate follow up investigations may eventually lead to

complacency and unresponsiveness. But the lack of cVDPV circulation confirms the existence of strong immunization systems in the countries where these VDPVs were detected. Despite the high genetic divergence from Sabin, the VDPVs detected in Israel were found to be sensitive to isoflavenes antivirals.

Laboratory Quality Assurance Programme

i. Accreditation Programme

In the European region, 98% of the laboratories were fully accredited by WHO as of December 2010. The laboratory with particular concern is located in Uzbekistan where local regulations do not allow referring samples to RRL for confirmatory testing and intratypic differentiation (ITD).

ii. Proficiency Tests

A proficiency testing (PT) programme for the GPLN is coordinated by WHO in collaboration with two Global Specialized Laboratories (GSL) in the United States and the Netherlands. Six different PT panels are now in use for evaluating (i) accuracy of virus isolation (two versions, for the old and new virus isolation algorithms); ITD by (ii) ELISA, (iii) probe hybridization, (iv) traditional PCR, (v) realtime PCR (rRT-PCR), and, (vi) rRT-PCR for VDPV screening (newly introduced in 2010).

One national laboratory did not achieve a passing score for the isolation PT in 2010, the follow-up with this laboratory is on-going. Laboratories that attempted ELISA PT in 2010 attained passing scores of $\geq 90\%$. All laboratories that attempted the traditional PCR PT attained passing scores of $\geq 90\%$. All laboratories that attempted the two rRT-PCR panels for ITD and VDPV screening attained passing scores of $\geq 90\%$.

Several elements of an accreditation programme for sequencing laboratories are now available for use. The CDC-developed standard poliovirus sequencing protocol 2010 and the sequencing laboratory accreditation checklist 2010 have been pilot tested in the GPLN in 2011. A section on the sequencing of heterotypic mixtures (mixtures of more than one serotype) using specific primers will be added to the Standard Operating Procedure (SOP) in May 2011, and the complete SOP will be distributed for evaluation by four sequencing labs that serve areas with wild poliovirus circulation. The SOP will be updated based on their feedback and will be distributed to other RRLs and GSLs. The proficiency testing program will be piloted in 2011, without a requirement for scoring of performance.

iii Protocol for authenticating the identity of cell lines

The GSL in the United Kingdom has developed a standardized procedure for authenticating the identity of cell lines used for PV isolation in the GPLN. This procedure is based on sequencing a conserved mitochondrial gene fragment (COXA1) and performing rRT-PCR with melt curve analysis. The testing of cells from GSLs will begin in 2011.

iv. Rendering PV isolates non-infectious prior to shipment

The European region has pioneered the use of commercially available FTA cards for shipping nucleic acid from cells for *Mycoplasma* testing. The GPLN has evaluated FTA cards for

rendering virus isolates non-infectious prior to shipment for ITD and sequencing. Field trials in 4 labs showed that comparable ITD and sequencing results could be obtained for isolates shipped in the conventional manner and using the evaluated commercial product. Field tests are continuing in a few additional laboratories.

v. Biosafety Campaign

Participants learned of the development, pilot testing and distribution of training materials for a biosafety campaign that was launched in the GPLN in 2010. A "Training of trainers" workshop for biosafety focal points was held on Wednesday. Meeting participants learned how to use the training materials, which includes the need to ensure the use of both written training materials and videos. Sets of DVDs were sent to each laboratory.

Incorporation of rRT-PCR into AFP Specimen Processing Algorithm

The GPLN recommended the adoption of shorter algorithm for virus isolation in cell culture and an rRT-PCR algorithm for ITD and VDPV screening in June 2009, following field evaluations that showed higher sensitivity for VDPV detection than had been the case with previous algorithms. The European region has not adopted the new algorithm but is interested in developing an algorithm that will retain the ability to isolate non-polio enteroviruses while speeding up poliovirus detection. In the Tajikistan outbreak, initial results were timely, but later results were delayed because of the large number of specimens arriving in batches. Participants discussed the possibilities for improving VDPV detection and achieving a reduction in laboratory reporting times. Options included changing the virus isolation test algorithm or altering the timing during analytical processes for shipping of isolates that grow in the L20B cell line to RRL or GSL for characterization. The agreed-upon solution will require a minor change in the cell culture algorithm in use in National Laboratories (NLs) and sub-NLs and will allow the continued detection of non polio enteroviruses. It will incorporate the use of real-time RT-PCR for ITD and VDPV screening or the use of PCR followed by sequencing for the same purpose in the RRLs/GSLs that receive referred isolates.

To improve the efficiency and accuracy of testing it is recommended that the RRLs and GSLs discontinue the use of ELISA as an ITD method and replace it with use of rRT-PCR procedures. For settings where non-polio enterovirus characterization is a local requirement, it is recommended to re-position serotyping in the testing scheme to after rRT-PCR and restrict it to characterizing non-polio enteroviruses only.

Status of the rRT-PCR implementation:

- Trained personnel are now available in 4 laboratories.
- On-site training was provided for the Russian Federation in 2009 and the thermocycling equipment was provided by WHO. Testing of the PT panel from 2010 was delayed due to the Tajikistan outbreak.
- Other RRLs do not need a workshop or onsite training but may need assistance with implementation.

Supplementary surveillance

Over 40 Member States participate in AFP surveillance. With the exception of MECACAR countries, AFP surveillance is not up to certification standards. West, South and Nordic-Baltic sub-regions are of particular concern. In order to maintain certification-level surveillance standards, supplementary surveillance is essential.

Environmental surveillance for detection of PVs

Environmental surveillance for PV has been performed in some areas of the European region for decades. Twenty Member States have some environmental surveillance activities. The "WHO Guidelines for Environmental Surveillance of PV Circulation" have generally been followed when environmental surveillance is implemented. Environmental surveillance needs to be maintained and expanded in order to supplement AFP surveillance or to substitute for it where it is not implemented. The GPLN is exploring ways to improve the efficiency of analysis of sewage samples.

Enterovirus Surveillance

About 40 Member States report the use of enterovirus surveillance as a sole mechanism of polio detection or in conjunction with either AFP or environmental surveillance. The Regional office is currently working on the development of guidelines for enterovirus surveillance.

RECOMMENDATIONS:

1. For achieving a reduction in laboratory reporting times and improving VDPV detection in AFP specimens, the following is recommended:
 - a. RRLs are to use one of the following 3 options for ITD and VDPV detection
 - switch to rRT-PCR ITD and VDPV screening procedures and sequence only NSL isolates identified in either rRT-PCR test;
 - use one validated and quality assured ITD method (conventional PCR, probe hybridization or ELISA) and sequence all PV isolates;
 - sequence all poliovirus isolates, as recommended at the 2010 Informal Consultation of the GPLN (see meeting report at <http://www.polioeradication.org/ResourceLibrary.aspx>)
 - b. National labs will continue to do virus isolation in cell culture using the traditional algorithm and observation times. One of the possible changes is that L20B-positive isolates will be passed onto RD and then forwarded to the RRL when the cultures exhibit at least 75 percent CPE. National labs can continue to do serotyping by microneutralization in parallel to referring isolates to the RRL, if they wish, but serotyping will no longer be required prior to referring isolates or to performing ITD. Alternatively, all L20B-positive isolates should be referred to RRL without waiting for serotyping results.
2. At least 2 RRLs are to investigate the impact on virus detection sensitivity of using a shortened observation time for virus isolation (from 7 to 5 days) keeping all other elements of the traditional virus isolation algorithm unchanged.
3. The EURO lab coordinator should work with RRLs to prepare estimates of the amounts of rRT-PCR reagents needed for use in testing specimens from environmental and enterovirus surveillance. The information should be shared with WHO HQ and CDC to determine the feasibility of providing reagents.
4. International regulations for shipping of nucleic acid specimens should be disseminated to laboratories.

5. RRL directors need to familiarize themselves with the new online LDMS <http://ldms.euro.who.int> in order to initiate RRL reporting and support NLs in the implementation of the database according to the schedule for the Region.
6. RRL directors should use the recently developed WHO Biosafety training modules to assist in training lab directors in the NLs that are supported by the RRLs, as well as their own lab staff as needed. RRL directors should share the materials with Biosafety Staff at their respective institutions. RRL directors should give feedback to WHO about the training materials including need, if any, for developing modules on additional topics.