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**ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ
БЮРО**

Report of the Meeting of the Regional Reference Laboratories (RRLs)

WHO/Europe Polio Laboratory Network

Chamonix, France

24-25 June 2012

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 Chamonix, France with representatives from 8 member laboratories and WHO laboratory scientists with responsibility for coordinating the network's activities.

During the meeting, Lab Directors provided updates on their testing, research and training activities over the past year. The Lab Coordinator provided an overview of the performance by GPLN laboratories and activities in the Region. A total of 129,142 specimens from all surveillance activities were processed in the European region during the period January – December 2011. Of those, 3,188 were faecal specimens from cases of Acute Flaccid Paralysis (AFP). No wild-type polioviruses (WPVs) were detected during 2011 following last year's outbreak in Tajikistan. Challenges to improve timeliness of detection of WPVs and vaccine-derived polioviruses (VDPVs) were noted particularly those concerning the shipment of stool samples and poliovirus (PV) isolates to National Labs (NLs) or RRLs.

Performance of the Polio Laboratory Network in the European region in 2011 remained high although labs in Portugal and Spain underwent major assessments following poor results in PT panels and cell sensitivity assays identified during the 2010 accreditation process. An update was given on the ITD rRT-PCR methods capacity in RRLs and plans for the introduction of standardization and quality assurance procedures for sequencing methods. The use of the

Laboratory Data Management System (LDMS) has been successfully implemented by most labs following last year's introduction although some countries are still not reporting through this system. Recommendations were made to improve the quality assurance of enterovirus (EV) surveillance, the reporting of results from all types of surveillance activities, the links between RRLs and their associated NLs and the implementation of updated ITD rRT-PCR and sequencing methods.

SUMMARY OF DISCUSSIONS

1. Surveillance for poliovirus in the European Region

Performance of the Polio Laboratory Network in the European Region in 2011 remained high with 98% of isolations of polioviruses (PVs) and 99% of ITD results produced/obtained within the required timelines. A number of issues affecting or with potential effect on timelines for PV detection were underlined such as the batching of stool samples before shipment, the quality of samples and packaging, the delayed or incomplete communication of sample delivery and the strict regulations for shipping/handling of biological materials in some countries. One of the possible solutions to these problems is the implementation of ITD capacity in selected NLs although this should involve a careful evaluation to justify the investment. The use of FTA cards for the shipment of RD cultures will also ease the full characterization of PVs within required timelines. Detailed protocols for the preparation and processing of FTA cards (with video support) are available from the Regional Office (LDMS webpage <http://ldms.euro.who.int> ; videos accessible after successful log in). FTA cards should be stored and disposed using the same rules as for infectious waste.

Despite the excellent performance in the Region, some concerns remain for countries where no isolation of PV or non-polio enterovirus (NPEV) has been reported in the last few years. Changes in funding trends for the Global Polio Eradication Initiative (GPEI) and their possible impact on public health policies concerning virus surveillance and immunization strategies were discussed.

Other activities during this period included training of 3 virologists supported by WHO EURO and the organization of workshops on laboratory biosafety and biorisk management for polio labs. Four projects to assess new methods were also coordinated.

2. Supplementary surveillance

The European Region of the GPLN prioritizes the isolation and characterization of polioviruses from faecal specimens from AFP cases, supplemented by other surveillance techniques such as environmental (ENV) or enterovirus (EV) surveillance in its support of the GPEI. Laboratories in this Region have a broad range of experience and laboratory techniques. 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 programs. For this reason, it is recognized that the Region would benefit from the use of common guidelines, improved reporting requirements and a quality assurance program to support these surveillance activities, similar to the systems in use for AFP surveillance. These control systems should help identifying quality indicators to assess the effectiveness of these supplementary surveillance activities and include information on the type of clinical syndromes and specimens analyzed, timelines for the processing of samples and virus isolations and the geographical locations where sampling is taking place. Ideally, reporting of these activities will include the total number of samples analyzed including specimens with negative results. Scientists from RRLs can provide technical support for these activities regardless of them being conducted at the RRL or not.

2.1 Enterovirus Surveillance

About 40 Member States report the use of enterovirus surveillance as the only mechanism of polio detection or in conjunction with either AFP or ENV surveillance. It is good news that only 3% of the samples analyzed through EV surveillance in 2011 are not stool specimens since these are the best samples to confirm infection of PV in humans. However, this percentage is not the same across countries with stool samples representing less than 50% of the total of EV surveillance specimens in some countries. The Regional Office has prepared guidelines covering different aspects related to EV surveillance such as the rationale for EV surveillance, recommended procedures for specimen transport, EV detection and characterization (including laboratory protocols) and documentation and reporting of results. Draft guidelines have been prepared and are awaiting in-house clearance.

2.2 Environmental surveillance for detection of PVs

ENV surveillance for PV has been performed in some areas of the European region for decades. 20 Member States have some ENV surveillance activities. The "WHO Guidelines for ENV

Surveillance of PV Circulation" have generally been followed when ENV surveillance is implemented. The need to update the guidelines to reflect the latest detection methods has also been discussed. ENV 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.

3. Seroprevalence studies

Seroprevalence studies can provide useful information on the immune status of the population in some areas/countries. Results from these studies might help estimating the potential for WPVs and VDPVs to circulate and identifying relevant immunity gaps in certain geographical areas or specific age groups. These studies will become more relevant during the GPEI endgame to help monitoring the effect of ceasing OPV use and/or any other changes in polio immunization strategies. Differences in results between countries are likely to vary depending on national immunization policies and vaccine coverage.

4. Detection of Vaccine Derived Polio Viruses (VDPVs)

VDPVs have been isolated from various sources in the European region although none from AFP cases. 3 VDPV1 isolates (12-14 mutations) from a healthy child sampled at random in a kindergarten and 5 VDPV2 strains (16-18 mutations) from an immunodeficient child were isolated in Turkey. Similarly, 1 VDPV2 strain (14 mutations) was isolated from an immunodeficient child in Italy who later died from a respiratory infection. 1 VDPV2 was isolated from an immunodeficient child from Bethlehem (Palestinian Authority) who was being treated in an Israeli Hospital. A number of positive stool samples were obtained from this child before he died from non-virus related causes. The VP1 of the virus diverged by 1.2%. 1 VDPV2 isolate was obtained from the well known long-term immunodeficient excreter in the UK who has now been excreting PV for an estimated 26 years. VDPVs have been periodically found in ENV samples from European countries such as Finland, Estonia and Israel during the last several years. During 2011, 18 highly diverged VDPV2 strains from 15 sewage samples representing two separate excretors were collected from various collection points located throughout the greater Tel Aviv (Israel) sewage system. 17 VDPV2 isolates from 14 samples were from one epidemiological cluster (first isolate was obtained in 1998). VP1 divergence ranged between 15.9% to 16.3%. 1 VDPV2 isolate from 1 sample was from a separate epidemiological cluster (first isolate was obtained in 2006). VP1 divergence was 11.18%. Prolonged VDPV detections

remain a concern because of their potential of VDPVs to circulate in communities with low immunity. A study involving the spiking of Sabin virus into the sewage system is in progress in Israel with encouraging preliminary results that might help determining the limits of detection of the ENV sampling process. A world-wide study involving laboratories of the Pasteur Institute Network is assessing the prevalence of non-polio HEV-C viruses in human and ENV samples. These studies might help finding links between non-polio HEV-C isolates and cVDPV strains by identifying closely-related non-structural genomic sequences. This could eventually lead to the description of risk factors associated with increased potential for the circulation of cVDPVs in different populations. Results presented by the RRL in Russia showed a high proportion of false positives in VDPV rRT-PCR assays of 2011 PV isolates, particularly for type 2 PVs. An excessive proportion of false VDPV detections could result in increased workload and overuse of valuable resources. Scientists at CDC are currently trying to optimize protocols to improve the specificity of these assays.

5. Laboratory Quality Assurance Program

5.1 Annual accreditation process

The annual proficiency testing (PT) and assessment of laboratories continue to be critical for the quality assurance of the performance in polio labs. Six different PT panels are 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. The PT program is coordinated by WHO in collaboration with two Global Specialized Laboratories (GSL) in the United States and the Netherlands. Laboratories that attempted ELISA PT in 2010 attained passing scores of > 90%. All laboratories that attempted the traditional PCR PT attained passing scores of > 90%. All laboratories that attempted the two rRT-PCR panels for ITD and VDPV screening attained passing scores of > 90%.

The annual accreditation process is completed by an extensive review of a standard accreditation checklist filled by all laboratories. The accreditation checklist covers all aspects related to the work in the laboratory including timelines for sample processing and virus isolation, characterization of PVs, details of personnel involved in testing, biosafety practices, PT results, data management, etc. The checklist is divided in sections that are scored by the reviewer to give a final percentage score that will determine the accreditation status of the

laboratory. Accreditation visits to laboratories with performance concerns are conducted when necessary. In the European region, all WHO polio laboratories except one (Uzbekistan) were fully accredited by WHO as of December 2011.

5.2 Visits to NPLs Spain and Portugal

Following suboptimal results for cell sensitivity tests and the PT for 2010, NLs in Spain and Portugal underwent extensive assessment by inspection visits from representatives of two RRLs as proposed and organized by the Regional Office. The assessment visits revealed important deficiencies in laboratory procedures and training of personnel. A number of corrective measures to ensure effective PV detection in those countries were enforced immediately and recommendations were made to try to restore adequate performance in both NLs. It was also perceived that some of the recommendations such as the need to establish large cell banks to reduce requests for cells to RRLs could be extended to other NLs. The experience in Spain and Portugal highlighted the critical role of PT and cell sensitivity evaluation in identifying laboratories with performance deficiencies. The importance of using SOPs in accordance with WHO protocols for routine testing of samples by isolation and typing in cell cultures was also emphasized as these methods are fully validated and supported by WHO as they are also thoroughly evaluated through annual PT. It is possible that the fall in performance occasionally observed in few NLs can be prevented by RRLs working closer together with their associated NLs and scientists from RRLs being directly involved in the accreditation process of NLs.

Recommendations to improve training procedures for new personnel particularly for new laboratory heads were discussed. The need to continue using Biosafety WHO videos as part of the training was also emphasised. Laboratories should also be reminded of the importance of maintaining immunisation levels against polio for all personnel involved in laboratory work.

5.3 Authentication of cell lines used in the GPLN

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 fragment at the 5' end of the cytochrome c oxidase subunit 1 gene in the mitochondrial genome and performing rRT-PCR with melt curve analysis. The method is very sensitive and can detect the presence of 1 human cell in a background of 10^5 mouse cells.

NIBSC has tested L20B and RD cells from GSLs and all were found to be of the expected origin. Cell authentication testing will be expanded to all labs distributing cells to labs in the GPLN.

6. Laboratory Methods

6.1 rRT-ITD PCR of PV isolates

Results from the CDC GSL indicate that ITD sensitivity can be increased significantly by using a dual-stage (Rocket) rRT-PCR procedure which essentially consists in performing an initial set of 15 rRT-PCR cycles in more permissive conditions before the standard 40 cycle-ITD rRT-PCR reactions. This technique will be especially advantageous for high volume labs which are most likely to have serotype mixtures or samples with low virus concentration. There will be no need to extract RNA, thus saving time and cost. No change in current PV ITD rRT-PCR kits will be required. It is highly recommended that "Rocket" ITD be used if testing ENV samples with any of the ITD assays. Implementation of the Rocket ITD rRT-PCR would require validation of the technique for the different RT-PCR platforms used across polio laboratories.

Despite several attempts by laboratories of the GPLN, no progress has been made in the development of methods for the direct detection of PV from stool samples using PCR based assays. However, it is not easy to interpret negative results and how these can be avoided. Experiments in Israel have shown the value of using bacteriophage MS2 as control for RNA extraction from stool samples to detect inhibitors for PCR assays and to compare several RNA extraction kits available commercially. Results clearly showed differences between extraction techniques and indicate that the use of MS2 could help developing efficient methods for the direct detection of PVs from stool samples or any other sample from the different surveillance activities.

6.2 Nucleotide sequencing of PV isolates

An accreditation program for sequencing laboratories has been pilot tested in 17 laboratories of the GPLN including 3 in the European region. No scores were assigned although 15 labs obtained sequences that were identical to the expected sequence of the PT panel RNAs. Individual feedback was provided to each lab. A standard poliovirus sequencing protocol and the sequencing laboratory accreditation checklist were developed by CDC. A section on the sequencing of heterotypic mixtures (mixtures of more than one serotype) using new primers specific for currently circulating type 1 and 3 WPVs has been recently added to the SOP. New

PT panels are planned for 2012. It is not clear how current methods compare between sequencing laboratories in the Region. Attempts to compare methods would be useful. A decision to fully adopt the CDC sequencing SOP including the use of the same set of primers and testing algorithms should be considered.

7. LDMS reporting system

The new LDMS reporting system introduced in the European region last year was reviewed in detail. The online LDMS is a web based laboratory data management system that supports sample registration, sample tracking, testing, quality assurance and reporting, online queries and specimen and case management. It integrates lab-based AFP, EV and ENV surveillance and supports case-based AFP surveillance by dynamically linking and exchanging lab information with the AFP database. Together with the web-based AFP surveillance system, the online LDMS has provided the WHO European Regional Office with a potential platform for integrated polio surveillance. Linking of AFP data and stool sample data increased from 10% in 2007 to 90% 2011. Reporting through LDMS also helps minimizing reporting errors between surveillance and laboratory databases. It also allows the immediate notification of WPVs to WHO Europe and the tracking of stool samples in near-real time. Labs can also enter geographic information for ENV and EV samples. The LDMS is a comprehensive system that requires a minimum of data variables, it is very easy to use and update, it is available 24 hours a day, 7 days a week, has 3-tier security levels (RRL->NL->SNL) and allows for the quick validation of data.

The development of additional functionalities for LDMS was also discussed. A link of sequencing data with the RIVM EV typing tool database will soon be available. Specimen filter options will be added so that the current status of PV isolations can be reviewed and pending results identified easily. The use of LDMS should also contribute to the improvement of the completeness and timeliness of EV and ENV surveillance reporting. It will also be possible to use LDMS to record results and analyse data from different experiments and studies including those from quality assurance testing which would help alerting RRLs of possible deficiencies in their associated NLs. It will be possible to extract relevant information for example compare cell culture versus molecular based PV testing or evaluate the properties of different VDPV isolates.

Difficulties in obtaining full details of surveillance activities from laboratories not reporting through LDMS and/or not performing AFP surveillance were noted. Attempts to at least obtain annual aggregated data from those labs should be made. RRLs linked to labs conducting those surveillance activities could enter these results in the LDMS. Reports should

include the number of positive samples, total number of samples and breakdown by administrative division if possible. The importance of reporting through LDMS every week regardless of the presence of actual testing activities or not was indicated.

RECOMMENDATIONS:

1. RRL directors should make efforts to strengthen links with associated NLs to ensure the fast shipment of isolates and the early detection and management of performance concerns. To achieve this:
 - a. Communication between RRLs and their associated NLs should be improved to allow the timely information and follow-up of sample deliveries and to ensure the efficient shipment and receipt of PV isolates for characterization.
 - b. RRL directors should be directly involved in the accreditation process of NLs. For that purpose, accreditation checklists should be made available to RRLs by the Regional Office.
 - c. RRL directors may suggest the need for accreditation visits of NLs when they find compelling reasons following the critical review of NL performance.
2. Reporting of AFP surveillance testing results by laboratories of the WHO Europe polio laboratory network using LDMS is mandatory.
 - a. Laboratories who still don't report through this system should do so as soon as possible.
 - b. The Regional Office can assist if problems in implementation are encountered.
 - c. Reports through LDMS should be sent every week. When no testing activities have occurred, simple log in and log out is sufficient to record a zero report.
3. There is a need to identify quality indicators and to improve standardization procedures 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. Guidelines for EV surveillance developed by the Regional Office should be approved and distributed.
 - 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. Reporting of results from all types of surveillance through LDMS should be encouraged and to that end, new functionalities should be included in the LDMS

system to help improving the completeness and timeliness of EV and ENV surveillance.

- d. RRL directors should identify ways to support these activities whether they are directly involved in laboratory testing or not.
4. Laboratories with capacity for ITD rRT-PCR and nucleotide sequencing methods should participate in pilot and proficiency testing organized by WHO and the CDC to contribute to the improvement, standardization and quality assurance of these procedures.
 - a. The new ITD Rocket rRT-PCR method should be pilot tested in laboratories with rRT-PCR ITD capacity following instructions from CDC.
 - b. Sequencing labs should complete PT for sequencing for 2012 following instructions from CDC.
 - c. Protocols for sequencing methods should be compiled by the Euro lab coordinator to be shared between sequencing laboratories with a view to harmonize procedures and reagents.
 5. The annual evaluation of the quality assurance of activities conducted in all polio laboratories in the Region should continue to be a high priority.
 - a. Laboratories should review their training procedures to ensure an SOP exists with details of how competence for the different laboratory techniques should be acquired and evaluated.
 - b. Induction training for new laboratory heads of NLs should be planned and supervised by the director of the linked RRL.
 - c. Laboratories should establish large cell banks to minimize requests for shipment of cells from RRLs. A pan-regional request form for cell request that includes justification for request should be developed by the Regional Office.
 - d. The GSL in the UK should test the authenticity of RD and L20B master cell stocks from labs distributing cells to NLs.
 - e. 2-3 representatives from RRLs and GSLs should assist the accreditation process of NLs by carefully evaluating accreditation checklists.