3-4 September 2015 // Antwerp, Belgium

Meeting of the Global Polio Laboratory Network (GPLN) in the WHO European Region

World Health Organization
REGIONAL OFFICE FOR Europe

KEYWORDS

POLIOMYELITIS – diagnosis, epidemiology, prevention and control

POLIOVIRUS VACCINE – administration and dosage

LABORATORIES

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Abbreviations

AFP	acute flaccid paralysis
bOPV	bivalent oral poliovirus vaccine
BSL	biosafety level
CDC	United States Centers for Disease Control and Prevention
cVDPV(2)	circulating vaccine-derived (type 2) polioviruses
EV	enterovirus
GAPIII	*WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use*
GPLNMS	Global Polio Laboratory Network Management System
LDMS	Laboratory Data Management System
NPEV	nonpolio enterovirus
ITD	intratypic differentiation
NRAc	National Regulatory Authorities for Containment
OPV	oral poliovirus vaccine
PT	proficiency testing
PV	poliovirus
(qPCR)	qualitative real-time PCR
RCC	Regional Certification Commission
rRT-PCR	real-time reverse transcription-polymerase chain reaction
SOPs	standard operating procedures
tOPV	trivalent oral poliovirus vaccine
VDPV	vaccine-derived poliovirus
WPV (1, 2 or3)	wild poliovirus (type 1, 2 or 3)

Summary

Representatives from 45 of the 48 laboratories of the Global Polio Laboratory Network (GPLN) located in the WHO European Region (the European Regional Polio Laboratory Network) met with representatives from WHO headquarters and international partner agencies in Antwerp, Belgium from 3-4 September 2015. The main objectives of the meeting were to review and discuss the laboratory containment requirements described in the *WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use* (GAPIII), and to outline and discuss regional implementation of the revised algorithm for poliovirus (PV) testing. Additional objectives included updating the European Regional Polio Laboratory Network (the Network) representatives on:

- recent progress towards global eradication of poliomyelitis, particularly with regard to the current situation in the WHO Eastern Mediterranean Region and developments in the WHO European Region;
- introduction of the new Global Polio Laboratory Network Management System (GPLNMS);
- development of real-time reverse transcription-polymerase chain reaction (rRT-PCR) assays for intratypic differentiation (ITD) testing and vaccine-derived poliovirus (VDPV) screening;
- a review of recent changes in proficiency testing results and new developments in the polio eradication programme, and
- an outline of quality assurance procedures for cell sensitivity and mycoplasma testing.

Session 1: Global Action Plan (GAP III) biorisk management

According to the World Health Assembly resolution (WHA 68.3) adopted on 25 May 2015 all Member States are now required to implement appropriate containment of type 2 wild polioviruses (WPV) in essential facilities by the end of 2015 and of type 2 Sabin polioviruses (PVs) within 3 months of global withdrawal of the type 2 component in oral poliovirus vaccine (OPV) expected in April 2016. As of April 2016 it is expected that global trivalent oral poliovirus vaccine (tOPV) use will be replaced by bivalent oral poliovirus vaccine (bOPV) use. The five criteria to be met for global withdrawal of tOPV include:

1. all Member States will have included at least one dose of IPV into their immunization schedules;
2. all Member States continuing to use OPV will have access to bOPV that is licenced for routine immunization;

3. all Member States will have implemented global surveillance and response protocols for type 2 PV (including constitution of a stockpile of monovalent OPV type 2);
4. all Member States will have completed phase I PV containment activities, with appropriate handling of residual PV type 2 materials;
5. verification of global eradication of WPV type 2 will have been completed.

The trigger for setting a date for global withdrawal of type 2 OPV will be the absence of all persisting circulating vaccine-derived type 2 polioviruses (cVDPV2) for at least 6 months.

GAPIII was published in 2014[1]. This action plan aligns the safe handling and containment of PV infectious and potentially infectious materials with the WHO Endgame Strategy[2], and describes timelines and requirements to be completed in preparation for poliovirus type 2 containment, implemented throughout the PV type 2 containment period, and applied in the post-eradication and post-bOPV phase. It also addresses type-specific containment of WPV as well as OPV/Sabin PVs, consistent with the goal of sequential cessation of OPV use after type-specific WPV eradication.

GAPIII will be implemented in three phases, linked to national and international milestones in polio eradication. During Phase I Member States are required to conduct national laboratory surveys and establish WPV type 2 (WPV2) inventories, destroy unnecessary WPV2 materials, designate ***essential poliovirus facilities*** and transfer necessary WPV2 materials to these essential poliovirus facilities. Essential poliovirus facilities are expected to implement GAPIII (Annex 2 and 3) and demonstrate that appropriate and validated risk-reduction procedures have been established and implemented, and are continuously improved.

The plan calls for all WHO regions to identify OPV2/Sabin 2 infectious and potential infectious materials, and either destroy, transfer or contain the WPV2 materials by the end of 2015. All OPV2/Sabin2 materials should be destroyed, transferred or contained by July 2016. Regions have been requested to reduce the number of facilities containing poliovirus, as all essential poliovirus facilities will be required to demonstrate that appropriate and validated risk reduction procedures have been established and implemented.

It has been proposed that National Regulatory Authorities for Containment (NRAc) should certify all remaining PV facilities according to the requirements of GAPIII, and that the certification reports be

[1] *WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use (GAPIII)*. Global Polio Eradication Initiative 2014. Available online at: http://www.polioeradication.org/Portals/0/Document/Resources/PostEradication/GAPIII_2014.pdf
[2] *Polio Eradication and Endgame Strategic Plan (the Endgame Strategy) 2013-2018*. Global Polio Eradication Initiative 2014. Available online at: http://www.polioeradication.org/Portals/0/Document/Resources/StrategyWork/PEESP_EN_A4.pdf

submitted to the Regional Certification Commission (RCC) for evaluation. It is essential, therefore, that all Member States have established and have functional NRAcs. A containment certification scheme is currently in draft form and is expected to be finalized in the near future. This scheme describes the roles and responsibilities of the various stakeholders, including the facilities themselves, NRAcs, WHO and the international oversight bodies.

Meeting participants were provided with a condensed version of the GAP III training course that described the requirements, potential impacts and timelines of the Plan, and introduced the underlying biosafety and bisosecurity management system principles and concepts.

Discussion

It is essential that the PV diagnostic capabilities of all Network laboratories be maintained, but they will not all belong to essential poliovirus facilities. The ability of diagnostic laboratories to receive and test specimens, to conduct required quality control and quality assurance activities, and to be proficiency tested, will be maintained. Progressive changes within the Network, such as changes to the testing algorithm and to proficiency testing systems, are bringing the Network into line with the new containment requirements. Longer-term changes, such as the progression towards molecular detection of PVs, will further remove the requirement for polio laboratories to use live poliovirus materials.

The containment requirements outlined in GAPIII are based on a risk-management approach (Annexes 2 and 3), rather than on attempting to ascribe a set level of risk presented by PV. Current international biosafety level (BSL) recommendations, developed to handle pathogens of differing levels of risk, are interpreted and applied differently in different countries. It is unlikely that facilities currently operating standard BSL-3 conditions will be sufficient to handle polioviruses in future, but that containment conditions required will be dependent on a detailed risk assessment.

Many countries already have well-established laboratory risk assessment protocols and risk mitigation requirements in place, but these are not standardized and may need to the revised in light of GAPIII requirements. An itemized template or algorithm for conducting a risk assessment, preferably as part of a laboratory quality assurance process, would be helpful, although past experience has suggested that developing a standard template appropriate for all circumstances would be difficult. Existing templates and draft templates already is use in the Region could be shared to help countries standardize their procedures.

Facilities not nominated as essential poliovirus facilities will be expected to destroy all infectious and potentially infectious materials, or render them non-infectious. GAPIII describes the range of

infectious and potentially infectious materials, which includes stool collections that have not been specifically tested to exclude PVs, including vaccine viruses. Laboratories working on non-polio enteric pathogens need to be made aware of the new requirements for containment of polio materials, including PV vaccine materials, and countries will need to establish systems to ensure compliance with the requirements.

Session 2: GPLN updates and implementation of the new algorithm

Overview of the Global Polio Eradication Initiative and status of the Global Polio Laboratory Network

We are currently approaching a polio-free Africa, having achieved a major reduction in detected cases during 2013 and 2014. In the first half of 2015 the only polio cases detected anywhere in the world were in Afghanistan and Pakistan, a total of 16 cases, all associated with WPV1. The last detected WPV2 case was reported in 1999, and the last detected WPV3 case was reported from Nigeria in November 2012. As the number of WPV-associated cases has declined, focus and concern has shifted to VDPV-associated cases. A new definition of cVDPV has been developed[3] and new operational response guidelines issued. Persistent cVDPV2 transmission has been detected in both Nigeria and Pakistan in the past 12 months, but the polio eradication programme is now on-track for the imminent elimination of these viruses, permitting the withdrawal of OPV2 and introduction of bOPV in April 2016. The detection and elimination of VDPVs has been greatly aided by the expansion of environmental surveillance activities in high-risk areas. There are currently 31 Network laboratories routinely involved in testing environmental samples. New guidelines[4] and a strategic expansion plan for environmental surveillance are available[5]. Whilst cVDPV2 transmission is being eliminated, other foci of VDPV transmission continue to be detected, most recently in Madagascar (VDPV1) and Ukraine (VDPV1), underscoring the need to move away from the routine use of OPV.

More than 200 000 stool specimens were processed in Network laboratories in 2014, and performance indicators remain good for all WHO regions. The number of laboratories conducting

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[3] *Reporting and Classification of Vaccine-derived Polioviruses*. Global Polio Eradication Initiative, July 2015. Available online at: http://www.polioeradication.org/Portals/0/Document/Resources/VDPV_ReportingClassification.pdf

[4] *Guidelines on Environmental Surveillance for Detection of Polioviruses*. Global Polio Eradication Initiative, Working draft March 2015. Available online at: http://www.polioeradication.org/Portals/0/Document/Resources/GPLN_publications/GPLN_GuidelinesES_April2015.pdf

[5] *Polio Environmental Surveillance Expansion Plan. Global Expansion Plan under the Endgame Strategy 2013-2018*. Global Polio Eradication Initiative, April 2015. Available online at: http://www.polioeradication.org/Portals/0/Document/Resources/GPLN_publications/GPLN_ExpansionPlanES.pdf

rRT-PCR has increased to 88 and all regions, with the exception of the European Region, have now introduced the new virus isolation and ITD testing algorithms. The European Region is in the process of introducing the new algorithms and expected to have the majority of laboratories using them by the end of 2015. Of the 146 laboratories in the GPLN, 143 are fully accredited and 3 are provisionally accredited.

Key GPLN projects for 2015 include roll-out of the GPLNMS, a web-based reporting and data management system, roll-out and further development of the rRT-PCR for ITD, continued development of the direct detection of poliovirus from stool specimens, pilot-testing of the new testing algorithm for environmental isolates and testing of new sampling devices for environmental surveillance. Another key project has been developing and implementing the biorisk training programme in support of GAPIII containment requirements.

The main challenges remain advocacy for GPLN needs and implementation of annual work-plan to sustain the momentum and maintain high-level of performance during the polio endgame.

Eastern Mediterranean Regional Polio Laboratory Network update

Many countries in the Region are facing issues due to conflict, civil unrest or insurgency. This places considerable constraints on maintaining the Regional Polio Eradication Initiative, but despite some serious challenges at country level, progress continues to be made. After experiencing setbacks in 2013 and 2014, due in part to importations and reinfections, WPV circulation in 2015 is again restricted to Afghanistan and Pakistan. Despite improvements in surveillance the number of detected cases shows a significant year-on-year reduction. Pakistan, in particular, has shown an 81% reduction in the number of cases compared with the same time period in 2014. Afghanistan is considered to be close to elimination, but sporadic cases have continued to occur, with 8 cases detected in the first 6 months of 2015. A dramatic decline has also been noted in genomic diversity of viruses isolated, with only 2 clusters of WPV1 belonging to 5 lineages detected in 2015. Some of the circulating lineages have only been detected through environmental surveillance.

Environmental surveillance in the Region was first established at selected sites in Egypt in 2000, and its use has since been extended nationwide in Egypt and to key sites in Afghanistan and Pakistan. Regional plans have been developed for further extension to additional sites in Afghanistan and Pakistan, and to Somalia, Sudan, Syria and other areas considered at risk of poliovirus importation.

All laboratories in the Eastern Mediterranean Regional Network are fully accredited and performance indicators remain high. There are concerns over the very high workloads currently being experienced by the laboratories in Egypt and Pakistan; and there are possible indications of

declining performance in some of the National Laboratories. The deteriorating security situation in Iraq and Syria is a major concern, as are continued difficulties in transporting specimens across international borders. As the polio endgame approaches, the demands to establish and maintain environmental surveillance in the Region continue to increase, threatening to overwhelm available resources.

European Regional Polio Laboratory Network update

All laboratories in the Network are accredited for 2015, and all have passed their respective proficiency tests. The Region now receives laboratory data derived from acute flaccid paralysis (AFP), environmental and enterovirus (EV) surveillance systems, data being collected through the Laboratory Data Management System (LDMS).

While it has been relatively common to detect VDPVs in past years, the most recent VDPVs in the Region detected in western Ukraine in 2015 have strong evidence for circulation. Two VDPV-associated AFP cases were detected towards the end of August 2015 and investigation of the cases and virus isolates obtained is ongoing. Although the quality of AFP surveillance in Ukraine has remained reasonably high, available evidence suggests the level of vaccine coverage has dropped to below 50% in several parts of the country, particularly western areas.

Regional guidelines on EV surveillance have now been published[6], and a new algorithm for molecular typing of EV isolates has been developed. An example of an online EV typing tool, providing phylogenetic analysis of isolates, was also presented.

As traditional poliovirus typing sera will no longer be available, a new algorithm for poliovirus isolation in Network laboratories has been developed, together with an algorithm for molecular ITD of L20B-positive isolates. 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 and reduces the period of observation from 7 to 5 days per passage following addition of the stool extract to a cell culture. All L20B-positive samples are tested by molecular assays using real-time PCR to identify virus-positive cultures as non-polio EV (NPEV) and/or PV. PVs are classified as Sabin-like, possible WPV or possible VDPV. Off all programmatically important PVs at least a part of VP1 is sequenced.

[6] *Enterovirus surveillance guidelines. Guidelines for enterovirus surveillance in support of the Polio Eradication Initiative.* WHO Regional Office for Europe, 2015. Available online at: http://www.euro.who.int/__data/assets/pdf_file/0020/272810/EnterovirusSurveillanceGuidelines.pdf

The recommended molecular ITD regimen requires a complex array of tests that will not be applicable in all Network laboratories and it is likely that some laboratories will need to send L20B-positive cultures to another laboratory for ITD. Three training workshops in molecular ITD are planned for 2016. The transition to use of the new virus culture and ITD algorithms by all Network laboratories by the end of 2015 remains the focus of attention.

Discussion

Investigation of the cVDPVs detected in Ukraine is ongoing, with contact stool collection and testing, and enhanced supplementary surveillance. The investigations are at an early stage and more results will be released as they become available.

The new algorithm for isolation of PVs is specifically targeted at polioviruses, and should result in more rapid detection of L20B cultures. The new algorithm has been extensively field-tested and shown to be 99% sensitive for poliovirus. None of the laboratories that have already switched to the new algorithm have experienced any problems in implementation. However, some laboratories are concerned that the new method may affect the quality of EV surveillance, which is an essential function for some. There is, however, no impediment to laboratories further investigating samples using in-house procedures outside their duties as WHO network laboratories. Laboratories that are using traditional PV-permissive cell lines and are engaged in diagnostic EV surveillance, rather than specifically poliovirus surveillance, should exclude the possibility of PV-presence in the samples under investigation by any of WHO recommended techniques. The molecular typing of enteroviruses (EVs), when applicable, should be recommended as an alternative approach to eliminate PV containment challenges linked with sample inoculation in PV-permissive cell lines.

Session 3: Development of new information platforms and new diagnostics

Global Polio Laboratory Network Management System (GPLNMS)

This online management system, devised by Novel-t Innovative Solutions in consultation with WHO, allows laboratories to provide annual reports by entering and updating laboratory data, details of laboratory capacities and laboratory indicator values in a web-based system. The system will also be used to generate laboratory accreditation reports that can be reviewed during the accreditation process. This will improve coordination between the different levels of the GPLN by capturing comprehensive laboratory data generated by the network allowing identification and correction of gaps in global management of laboratory information. It will streamline key processes used to

monitor the quality of laboratory performance such as annual reporting, accreditation and proficiency testing (PT).

Once a year, each laboratory is required to submit an annual report via the GPLN platform. To produce the annual report, two types of data are required from the laboratory: general information about the laboratory (e.g. staff, equipment, facilities, shipments) together with information on the laboratory's capacities (e.g. on-site reviews), and the indicator values, linked to the capacities of a laboratory. The annual report module was pilot-tested by 20 laboratories and their 2014 reports and requested feedback were provided by mid-March 2015. Pilot-testing was successful and the target is now to have all Network laboratories using the system to submit their annual reports for 2015.

A module for the entry of accreditation status has been developed and will be pilot-tested during 2015. A small number of laboratories from all regions are required to beta-test the accreditation online data entry module, and volunteer laboratories are actively being sought. 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.

rRT-PCR assays for ITD testing and VDPV screening: recent improvements and next steps

As global polio eradication gets closer, demands placed on the laboratories continue to evolve and priorities change. Protocols for ITD rRT-PCR are continuously being updated as there is a constant demand for improved sensitivity and specificity to rapidly 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 identifying and characterizing virus mixtures, particularly WPV in homotypic mixtures. Improved ITD rRT-PCR methods are routinely used for the characterization of PV isolates from environmental samples and will eventually be used for the direct detection of PV isolates from stool samples. Wild PV-specific rRT-RCR assays have been shown to be effective for detection of wild viruses in homotypic mixtures, as the assays do not detect Sabin viruses or VDPVs. These assays are particularly useful in the evaluation of environmental isolates, where mixtures of viruses are commonly encountered.

Laboratories in the European Region have contributed to the pilot-testing and validation of rRT-PCR ITD assay version 4.0 (ITD 4.0), which includes reactions for EVs/Sabin-multiplex, Pan-PV, WPV1-multiplex, WPV3-WEAF-B and WPV3-SOAS. Further improvements have also included the CODEHOP Pan-PV assay, optimizing probe concentrations to reduce background and redesigning WPV and Sabin 1 probes to eliminate cross-reactivity. Experience has shown that ITD 4.0 is more sensitive than previously available assays, with lower limits of detection, especially with mixtures, and that

test signals are easier to interpret. ITD 4.0 has fewer discrepant results as the wild-virus-specific assays have replaced the serotype assays, which are highly degenerate and sometimes give weak/negative results with virus mixtures.

Implementation plans for the rRT-PCR ITD 4.0 test have begun. Kits will include 100 reactions instead of the previous 50, and 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 may be added in late 2015.

With the significant decline in poliomyelitis cases due to WPV in recent years, cases due to VDPVs assume greater importance. The current rRT-PCR VDPV assays have some limitations as they are 'rule-out' assays that identify viruses matching Sabin viruses in the target regions. In homotypic mixtures the Sabin signal may mask the presence of a VDPV in these assays. Furthermore, it has been found that up to 50% of the Sabin 2 isolates recently detected in Nigeria do not match reference Sabin 2 in the target region, even though in all other respects they appear to be normal Sabin 2 viruses. To overcome these limitations new 'rule-in' rRT-PCR VDPV assays are being developed to directly target VDPVs. These assays take advantage of sites under strong selection for amino acid reversion, often encompassing or flanking antigenic sites, and use can be made of multiple probes to simultaneously target sites present in different viruses. These assays appear to be very good at detecting VDPVs from RNA extracted directly from stool specimens, but are not currently, and may never be, 100% sensitive. Evidence to date suggests >98% of known Sabin 1- and Sabin-2-derived VDPVs are detected. The main limitation at present is that both the 'rule-in' and 'rule-out' VDPV assays target the same amino acids.

The next steps include roll-out of ITD 4.0 to all Network laboratories conducting ITD. A further revision, ITD 4.1, will include adjustments to the WPV probes and primers used. Development of ITD 5.0 is underway and will include an assay to detect PV2 after the switch to bOPV. Further development of the rule-in assays for VDPVs is ongoing, as is work on finalizing methods for the direct detection of poliovirus from stool and environmental samples.

Session 4: Quality assurance

Viral Isolation Proficiency Testing: review and introduction of the new testing scheme

The virus isolation proficiency test includes a panel of 10 samples that are either negative or positive for PVs or EVs, as single or mixed isolates. Panels do not contain WPVs or VDPVs, and the panel distributed for 2015 will be the last to contain PV2. Laboratories are required to use WHO-recommended testing methods and report results within 14 days of receipt of the panels. The passing score for all laboratories is 90%, with penalties applied for isolation or typing errors. Application of the new detection and isolation algorithm will make it easier to validate proficiency testing as the system focusses on PV isolation without the need to type the isolates detected, and if the algorithm is correctly followed the risk of missing a PV is small.

Laboratories in the European Region were given the option to conduct the 2014 PT for virus isolation following either the old or the new algorithm. A total of 46 of the 48 laboratories completed the proficiency test. Only one laboratory chose to use the new algorithm alone, 36 used the old algorithm and 9 a combination of both. A number of errors were detected, including errors in reporting, serotyping and failing to detect specific cell infections. The most problematic sample was sample 10, which contained a mixture of PV1 and echovirus 6. Three laboratories failed to pass the initial test, but all subsequently passed on retesting.

The panel for the 2015 PT is now ready for shipment and will be validated using the new virus isolation algorithm. It is essential, therefore, that all laboratories switch to use of the new algorithm before they receive the panels.

ITD proficiency testing: a review

ITD/VDVP PT panels consist of lyophilized *in vitro* non-infectious RNA transcripts of positive (genome-sense) polarity containing specified target sequences, including both 5'-NTR and VP1 sequences. Panels include 10 samples, in 4 unique panel versions, that should be tested following the molecular algorithm. They generally include PVs (singles and mixtures), NPEVs and negatives. A passing score is ≥90%, based on final reported result with deductions for technical issues, including a 15% deduction for failure to detect WPV or VDPV present in the sample. Additional deductions exist for misinterpretation and not correctly following the algorithm. When this scoring system was applied to the 2014 PT several laboratories attained low scores, mainly due to errors in interpretation of the new rRT-PCR protocol. Because of this it was decided to use a modified system to assign final PT scores with 'directed retesting or reanalysis of one or more samples in laboratories achieving lower scores'. Based on final results, 98% of laboratories tested achieved a passing score.

Following review of past problems encountered in laboratories, troubleshooting procedures recommended by the United States Centers for Disease Control and Prevention (CDC) should help

solve 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.

Distribution of the next ITD proficiency panel will start in November 2015, and it is intended that the new scoring system will be applied to all laboratories.

Sequencing proficiency testing: a review

The PT for sequencing requires the sequence analysis of PCR fragments containing the gene coding for VP1 capsid protein. RNA samples are sent to laboratories that are requested to amplify a region of approximately 1kb, including the VP1 region, using generic or specific PV primers to identify individual virus or components in a mixture. Laboratories then sequence the amplified PCR products, edit the results using specialized software and provide a final nucleotide sequence result.

PT for sequencing was introduced in 2011 with no scoring provided. 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, with five European laboratories scoring 100%, two laboratories scoring between 90% and 99% and one laboratory obtaining 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.

Common problems observed included sequence length or trimming errors, sequence insertion or deletion due to low quality sequence and editing errors, lack of understanding of appropriate use of the primers, problems with editing and documentation errors. Remedies to support laboratories failing the sequencing PT 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 be given the opportunity to repeat the PT during 2015.

Possible refinements in the PT process 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, including 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.

Cell-sensitivity/mycoplasma testing and investigation of cell contamination: a review

Laboratories are required to conduct cell sensitivity testing, to assure that cell cultures remain sensitive to poliovirus infection, by periodically titrating polio reference preparations of known titre. The sensitivity should be known for all frozen stocks of a cell line and should be evaluated whenever fresh cells are resuscitated or received in the laboratory. It is recommended that cells be evaluated approximately midway through their expected use of 15 passages. 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.

Both L20B and RD cells are required for poliovirus testing, and it is essential to prevent cross-contamination of these two cell lines. A very sensitive real-time PCR method has been developed to detect contamination of L20B cells with human cells and can be used for cell authentication of laboratory cell lines. This test has now been fully validated and is available for testing master and working cell banks from GPLN laboratories. The method can be expanded to include detection of other cells susceptible to poliovirus infection, such as cells from non-human primates. It is essential that laboratories supplying cells to other laboratories have their cell banks authenticated prior to distribution.

Mycoplasma are a frequent cause of contamination in cell cultures that may affect cell growth, cause chromosomal aberrations and induce changes in cell metabolism. They are not easily detected, even at high densities. Their small size allows them to pass through the 0.2 µm filters often used in cell culture technology and the absence of a cell wall confers β-lactam resistance. The current industry standard for mycoplasma testing employs traditional methods of culture on solid agar and broth, which is time consuming and relatively insensitive, and DNA staining using indicator cell lines, which can be non-specific, subjective and difficult to interpret. Both of these methods

involve growing and handling live mycoplasma, risking possible contamination of uninfected cell stocks. To overcome this problem a mycoplasma qualitative real-time PCR (qPCR) assay has been developed and validated. The assay is able to detect a broad-range of mycoplasma species, uses a single tube assay with an internal control, is rapid (the whole process takes 2-3 hours), has high specificity and sensitivity, and is robust. Using enriched spent medium, the performance of the qPCR assay is demonstrated to be comparable to (or better than) the conventional culture-based method (from Day 1 post-infection in both MRC-5 and WHO Vero cells). Both enriched spent medium and gDNA extracted from cultured cell pellet can be used for testing using the developed qPCR assay, with comparable performance. Using enriched spent medium or gDNA, the qPCR assay enables positive detection from day 2 following infection of MRC-5 and WHO Vero cultures (at low cell densities) with low titers of mycoplasma (~10 CFU) for all 5 mycoplasma species. For reliable detection, the minimum number of days prior to harvesting of samples for testing is 2 days following the last cell passage or medium change. Although this assay has been validated it has not yet been released.

Recommendations

1. The global switch to bOPV use will have profound consequences on laboratory containment requirements that will affect all members of the GPLN. In this respect laboratory staff should:
    - read through the GAPIII document in its current presentation to understand the implications for their laboratories and for their national certification requirements;
    - consider carefully if they need 'essential' laboratory status and are prepared to meet the stringent containment requirements;
    - raise concerns with WHO headquarters if there are technical requirements described within the GAPIII document they consider to be unreasonable, technically inaccurate or inappropriate;
    - ensure that they have the contact details of their National Regulatory Authorities responsible for containment and national agencies responsible for biosafety and biosecurity, as they will be required to work collaboratively with these authorities;
    - review the GAPIII requirements to see if there is some degree of flexibility within the guidelines that will permit them to meet the containment requirements without investing in expensive new facilities or equipment.
2. All laboratories in the Region should now adopt the new algorithm for PV isolation. This will require development of new standard operating procedures (SOPs), worksheets, reporting forms etc.

3. The WHO Regional Office for Europe will continue to work with laboratories to identify those most suitable for implementing the rRT-PCR ITD method, and will provide support to these laboratories through supply of kits and access to training.
4. All laboratories in the Region should be using the web-based GPLNMS to submit their annual reports, and any not yet doing so should move to this system as a matter of urgency.
    - A module for the reporting of accreditation results has been developed and volunteer laboratories are being sought by WHO headquarters to pilot test this system. Any laboratories in a position to test the new module should apply to the Global Polio Laboratory Coordinator.
5. The 2015 virus isolation proficiency test will be scored according to the new testing algorithm, and all laboratories should strictly follow this testing algorithm for completing the proficiency test.
    - Laboratories are requested to pay careful attention to the documentation process and avoid reporting errors.
    - Proficiency panels will soon be distributed and laboratory managers are requested to carefully check the provided *pro forma* invoice details in advance to ensure that the contact details are correct.
6. Maintaining high levels of laboratory quality assurance according to the WHO accreditation process is essential. To achieve this, all laboratories should:
    - use methods that have been standardized and validated by WHO for which PT evaluation is possible;
    - perform PT procedures following recommended instructions, which include reporting results on time and in the right format;
    - 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);
    - use Master and Working Cell banks that have been prepared from authenticated cell lines received from global specialized or regional laboratories.

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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