Training Workshop Report

Second Regional Hands-on Training Course
To Implement Real-Time Polymerase Chain Reaction Technique for Rapid Detection and Characterization of Polioviruses

03-07 December 2012
Manila, Philippines

World Health Organization
Western Pacific Region
REPORT

SECOND REGIONAL HANDS-ON TRAINING COURSE TO IMPLEMENT
REAL-TIME POLYMERASE CHAIN REACTION TECHNIQUE FOR RAPID DETECTION
AND CHARACTERIZATION OF POLIOVIRUSES

Convened by:

WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

Manila, Philippines
3–7 December 2012

Not for sale

Printed and distributed by:

World Health Organization
Regional Office for the Western Pacific
Manila, Philippines

April 2013
NOTE

The views expressed in this report are those of the participants of the Second Regional Hands-on Training Course to Implement Real-Time Polymerase Chain Reaction Technique for Rapid Detection and Characterization of Polioviruses and do not necessarily reflect the policies of the World Health Organization.

This report has been prepared by the World Health Organization Regional Office for the Western Pacific for the participants of the Second Regional Hands-on Training Course to Implement Real-Time Polymerase Chain Reaction Technique for Rapid Detection and Characterization of Polioviruses, which was held in Manila, Philippines from 3 to 7 December 2012.

This training was funded by the Korea Centers for Disease Control and Prevention, Republic of Korea.
SUMMARY

The Second Hands-on Training Course on Implementing Real-Time PCR Technique for Rapid Detection and Characterization of Polioviruses in the Western Pacific Region was held at the Research Institute for Tropical Medicine (RITM) in the Philippines from 3 to 7 December 2012.

The training was organized by the Expanded Programme on Immunization (EPI) of the WHO Regional Office for the Western Pacific, and was hosted by the Virology Department, RITM, Philippines.

The training session was attended by seven participants from six WHO-accredited national polio laboratories from Hong Kong (one participant), New Zealand (one participant), Philippines (two participants), Republic of Korea (one participant), and two laboratories in Viet Nam (two participants). In addition to WHO Secretariat, temporary advisers from the United States Centers for Disease Control and Prevention (US CDC), Victorian Infectious Diseases Reference Laboratory (VIDRL) in Australia attended as facilitators.

The objectives of the workshop were:

1) to enable participants to learn the new real-time PCR technique for rapid detection and characterization of polioviruses;

2) to enable participants to familiarize themselves with hands-on practice on real-time techniques for intratypic differentiation (ITD) of polioviruses and vaccine-derived polioviruses (VDPV) screening using real-time polymerase chain reaction (PCR) platform; and

3) to discuss problems and challenges for polioviruses laboratories and provided updates of the Global Polio Laboratory Network (GPLN) including new laboratory performance indicators.

The hands-on training, which consisted of lectures, country reports and practical sessions, focused on understanding the needs and role of the polio laboratory with function of performing intratypic differentiation and facilitating learning and training in the use of real-time PCR technique for rapid detection and characterization of polioviruses.

Overall, the participants were positive in their feedback and considered the workshop to have met the objectives and the schedule and administrative arrangements to be well organized.

The workshop participants were encouraged to contact each other and the facilitators after the workshop to ensure successful completion of four implementation steps, to be upgraded to polio ITD laboratories, and to obtain full proficiency in performing real-time PCR for ITD and VDPV screening.
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Keywords:

Poliomyelitis-prevention and control/Poliovirus/Polymerase chain reaction-utilization/
Health personnel-education/Vaccines/Laboratory personnel-education
1. INTRODUCTION

The WHO Global Polio Laboratory Network (GPLN), which is comprised of global specialized, regional reference, national and subnational laboratories, plays a crucial role in the global polio eradication initiative. The polio laboratory network in the Western Pacific Region consists of one global specialized laboratory (GSL) in Japan, two regional reference laboratories (RRLs) in Australia and China, and nine national polio laboratories (NPLs) one each in Malaysia, Mongolia, New Zealand, Philippines, Republic of Korea, Singapore, Hong Kong China and two in Vietnam and 31 subnational laboratories in China.

The regional poliomyelitis laboratory network played an important role in certifying poliomyelitis eradication in 2000 and in maintaining polio-free status by providing accurate and timely laboratory results of acute flaccid paralysis (AFP) samples in the Region. The performance of network laboratories is monitored through a laboratory accreditation programme established by WHO. Elements of WHO accreditation are proficiency testing, on-site performance reviews, monitoring of accuracy, and timeliness of reporting. All laboratories in the regional poliomyelitis laboratory network are performing according to WHO accreditation standards.

Despite continued good performance of the polio laboratory network in the Region, concern has been expressed about the challenges of maintaining certification standards for reporting and investigating AFP cases and collecting adequate stool specimens. There is concern that priorities may have shifted to other public health activities after certification and that complacency may have evolved following the interruption of wild poliovirus transmission in the Region.

Rapid confirmation of the transmission of wild polioviruses and vaccine-derived polio viruses (VDPVs) is an essential first step towards implementing interventions to prevent virus spread. GPLN has formulated a strategic plan to reduce laboratory reporting time without compromising poliovirus detection sensitivity by introducing the new algorithm for virus isolation and intratypic differentiation (ITD) of polioviruses. In the Western Pacific Region, the new test algorithm for virus isolation was introduced among network laboratories (excluding China subnational laboratories) during the period 2009–2010, and real-time polymerase chain reaction (PCR) for ITD of polioviruses and VDPV screening was introduced among five polio laboratories with ITD function. Virus isolation results are reported within 14 days of receipt of the samples, and ITD results are reported within seven days after virus isolation.

In 2009, the training course on implementing RT-PCR technique for rapid detection and characterization of wild polioviruses was organized and held in Melbourne, Australia. The objective of this training was to enable laboratory staff to learn the new real-time PCR technique for rapid detection and characterization of polioviruses and to discuss problems and challenges for poliovirus laboratories and provide some updates of the GPLN including new laboratory performance indicators. Participants from Australia, China, Japan, Malaysia, and Singapore attended this training. A second regional hands-on training course to implement real-time PCR technique for rapid detection and characterization of polioviruses was held in the Research Institute for Tropical Medicine (RITM) Philippines from 3–7 December 2012. The training introduced the new dual stage real-time PCR technique. Staff from two national polio laboratories with ITD function and staff from four national polio laboratories were invited to attend.
After the training, the six laboratories were equipped to perform the steps to gain proficiency of the techniques. Until the laboratories pass the proficiency test (PT), parallel ITD testing should be conducted in designated WHO polio GSL or RRLs in the Region.

1.1 Objectives

(1) to enable participants to learn the new real-time PCR technique for rapid detection and characterization of polioviruses.

(2) to enable participants to familiarize themselves with hands-on practice on real-time techniques for intratypic differentiation (ITD) of polioviruses and Vaccine derived polioviruses (VDPV) screening using real-time PCR platform and.

(3) to discuss problems and challenges for polioviruses laboratories and provide updates of the Global Polio Laboratory Network (GPLN) including new laboratory performance indicators.

1.2 Participants

The training session was attended by seven participants from six WHO-accredited national polio laboratories in Hong Kong (China) (one participant), New Zealand (one participant), Philippines (two participants), Republic of Korea (one participant), and Viet Nam (two participants). In addition to WHO Secretariat, temporary advisers from the US CDC and VIDRL in Australia attended as facilitators.

1.3 Opening Remarks

Dr Remigio M. Olveda of the Research Institute for Tropical Medicine, Philippines, and Dr Youngmee Jee of the WHO Regional Office for the Western Pacific welcomed the participants and delivered the introductory speech to open the hands-on training workshop.

2. PROCEEDINGS

2.1 Lecture sessions

2.1.1 Latest developments in the poliomyelitis laboratory network in the Western Pacific Region

Dr Youngmee Jee started the workshop with a presentation of the latest developments of the poliomyelitis laboratory network in the Western Pacific Region. The Expanded Programme on Immunization (EPI) in the Western Pacific Region have targeted disease control initiatives that include maintaining polio-free status and maternal, neonatal tetanus elimination in five remaining countries (Cambodia, China, Lao People's Democratic Republic, Papua New Guinea, and the Philippines) and two twin goals by 2012 namely: measles elimination and hepatitis B control. Dr Jee emphasized that to maintain polio-free status, there should be high coverage of polio vaccination (routine and supplemental immunization activities), sustained high-quality surveillance for AFP and performance of laboratory network, and laboratory containment of wild poliovirus. Notably, China has been polio-free for one year, with the last wild case detected on 09 October 2011. There has been remarkable progress since the 1988 World Health Assembly
(WHA) resolution to eradicate polio. It was noted that there have been no new cases in India for past 23 months since the last case of WPV detected on 11 January 2011.

In September 2012, the United Nations Secretary-General announced his full support for polio eradication and made it a top priority during the United Nations General Assembly special event on polio. WHO Executive Board declared polio eradication a programmatic emergency for global health on 21 January 2012 and requested the Director-General to rapidly finalize the polio endgame plan. The Polio Eradication and Endgame Strategic Plan 2013–2018 is a comprehensive, long-term strategy that addresses what is needed to achieve a polio-free world by 2018, eradication of wild poliovirus by 2014, cessation of oral polio vaccine (OPV) type 2 by 2015 or 2016, global certification by 2018, and finally, cessation of bOPV (types 1 and 3) by 2019 or 2020.

Dr Jee highlighted the threat of wild polio virus importation and circulating vaccine derived polio virus in sustaining a polio-free status of the region. In a 2011 risk assessment for wild polio virus importation in WPR countries, Cambodia, Papua New Guinea and the Philippines were considered to be at high risk category, while in the 2012 risk assessment, Papua New Guinea and the Philippines continued to be in a high-risk category and Cambodia in the medium risk. The Wild Polio virus type 1 outbreak in China in 2011 due to importation and effective control measures were highlighted. The VDPVs detected in China and Viet Nam and consequent threat of circulating VDPVs were also discussed.

Dr Jee elaborated on the polio laboratory network in the Western Pacific Region, consists of 43 laboratories (1 global specialised laboratory, 2 Regional reference laboratories, 9 National laboratories and 31 provincial laboratories in China). It is encouraging to note that there are 29 laboratories performing intratypic differentiation (ITD) and it is proposed that after the training, four additional laboratories will be performing ITD.

The Polio network laboratories were fully accredited by WHO as of 2012. The timeliness of reporting of results for virus isolation by the national polio laboratories is more than 90% and less than seven days for ITD by the regional reference laboratory. Timeliness of reporting for the rapid confirmation of the transmission of wild polioviruses and VDPVs is an essential first step towards implementing interventions to prevent virus spread. Sequencing laboratory accreditation will be conducted in 2013 for the seven ITD laboratories.

There were several activities related to strengthening the polio laboratory network conducted in 2011 and 2012 such as the training workshop for China polio laboratory network on the new algorithm for virus isolation and biosafety awareness in February 2012, and polio real-time PCR in March 2012, the Third Meeting on Vaccine-Preventable Diseases Laboratory Networks in the Western Pacific Region in Manila, Philippines, the 18th GPLN consultation in Geneva, polio ad hoc meeting in US CDC, polio sequencing workshop in US CDC, and biosafety awareness training conducted by the RITM national polio laboratory in the Philippines.

There were some issues presented such as: (1) the delay in shipping of polio isolates from the first VDPV case of Pasteur Institute, Ho Chi Minh City, to National Institute of Infectious Diseases (NIID) in Japan due to a shipping permit problem, therefore, the response was delayed; (2) funding gap to cover the increased cost to operate additional ITD laboratories to cover additional kits/reagents for real-time PCR; and (3) the increased workload to coordinate different categories of 43 polio laboratories (virus isolation, ITD laboratories, sequencing laboratories), in addition, 385 measles laboratories and 20 JE laboratories. To address these issues, action plans
were prepared including upgrading four NPLs (two in Viet Nam, one in Korea and one in the Philippines) to ITD laboratories by 2013 and involvement of the regional/global laboratory experts in the laboratory accreditation.

The Regional Office for the Western Pacific polio laboratory network will: (1) hold the second regional hands-on training for real-time PCR for ITD and VDPV, (2) facilitate a third round of real-time PCR training for eight remaining China subnational laboratories in 2013, (3) hold a WHO Regional Office for the Western Pacific laboratory network meeting for polio, measles/rubella and JE on 11 to 15 March 2013, (4) distribute new laboratory reporting format in 2013, (5) and improve on timeliness of reporting polio results from non-AFP samples.

Dr Jee discussed the objectives of the training and four steps to be followed by the participating laboratories in attaining and ensuring proficiency in real-time RT-PCR for ITD and VDPV screening.

2.1.2 Real-time PCR for poliovirus identification, intratypic differentiation and VDPV screening

Mr Brian Emery, Scientist, polio molecular diagnostic development team, Centers for Disease Control and Prevention (CDC), USA discussed the rationale of real-time RT PCR (rRT PCR) for poliovirus identification, intratypic differentiation and VDPV screening that was developed to replace the conventional PCR. rRT-PCR has high clinical sensitivity, has high analytical specificity, can process either RNA or cell culture samples, can be performed on samples with a mixture of viruses with more rapid results, is easier to scale for larger number of specimens, and reduces chances of assay contamination. The real-time assays for ITD, VDPV screening and genotype were enumerated. Real-time ITD assays have the same rationale, targets and number of assays as conventional ITD PCR assays. PCR assay bench work is nearly identical to and has the same reporting format as rRT-PCR. However, rRT-PCR assays have new reagents, new machines, new analysis, and new troubleshooting. Its evaluation was completed with thousands of poliovirus isolates and the method has been evaluated on different real-time platform. Parallel testing and implementation in US CDC diagnostic laboratory was completed with 87 laboratories certified in the network. The algorithm of both ITD assays was also described.

The real-time VDPV screening assay is a new assay designed to replace enzyme-linked immunosorbent assay (ELISA) ITD. The reagents and assays are analogous to real-time ITD assays, its rationale is similar to Sabin ITD PCR assay, and reporting is similar to ELISA. From July 2009–August 2011, there were several vaccine-derived polioviruses (VDPVs) detected from AFP cases and from the environment. The ELISA ITD method is replaced by real-time VDPV assay because there might be some VDPVs that have been missed due to the problems of the method. VDPV assays are used to screen possible VDPVs by targeting a “key” antigenic site in VP1 (S1, amino acid #99; S2, amino acid #143; S3, amino acids #285-291). Mr Emery also discussed the algorithm of the new real-time VDPV. The evaluation was completed with more than 1000 poliovirus isolates and the parallel testing with ELISA has shown higher sensitivity for cVDPV detection for serotype 2 and equivalent for serotypes 1 and 3. Field evaluations were completed and 87 laboratories were certified in the rRT-PCR ITD assay and rRT-PCR VDPV assay.

2.1.3 Introduction to Applied Biosystems (ABI) 7500
Mr Brian Emery made comparisons between conventional PCR and real-time PCR by discussing the use of probes (Taqman) for real-time PCR and how both assays work. The real-time PCR has high sensitivity and specificity, as shown by the different amplification plots and standard curves. Real-time PCR can use RNA/virus isolates and the virus mixture. The assay reduces contamination by preventing PCR product carry-over. It is fast and can perform large scale samples and detects VDPV.

Mr Emery also discussed the application of real-time PCR for routine poliovirus diagnostics and discussed the different application of the technique: Its use in detection of multiple viruses in a sample by a single reaction, and experiments to substantiate the stability of the kit for detection of enteroviruses when stored at room temperature.

The changes for using the real time PCR assay in the ITD laboratory were discussed. The changes included the reaction set-up, result analysis and data storage. The conventional PCR is analysed through gel electrophoresis, while, real-time PCR is analysed through the amplification plot. In terms of data storage, conventional PCR has hard copy, gel photo, and sample list, whereas, real-time PCR has data storage in computer/CD, graphics, and sample list as well as hard copy.

Mr Emery also discussed the routine ITD laboratory work for setting up the rRT-PCR assay such as the reagent and sample set up, machine operation and data analysis and report. Sample set up includes sample processing and sample worksheets, reagent preparation and set up and plate layout. The ABI 7500 Real-time machine and software operations were described in detail.

2.1.4 Introduction to ABI data analysis

Mr Brian Emery discussed steps in analysing the data and reporting of results using ABI 7500 machine and its software. He emphasized checking the positive and negative control first to validate the assay, then, checking each sample against the negative control. If the curve is too low, the Y axis has to be changed. The succeeding steps for the analysis were demonstrated. The baseline or threshold should be adjusted and this can be changed to obtain the best baseline. An alternative way to analyse results is to use the multicomponent plot and raw data plot. In multicomponent plot, the data plot of the sample value is compared against the negative control data plot. A positive sample will be easily seen as a line above that of the negative data plot. Raw data plot is done by moving the bar in the show cycle box and the raw data plot of the sample value is compared against the negative raw data plot. The data can also be shown in a multiple plot view. Report data is generated as a Microsoft PowerPoint file. If there is no Microsoft PowerPoint software on the computer, the print screen function may be used to obtain the screen shot of the graph. Then the screen shot may be pasted in the Microsoft Paint program and the graph of rRT-PCR may be printed in hard copy. The ITD data interpretation was also discussed.

2.1.5 VIDRL’s experience with real-time RT-PCR for ITD and VDPV screening

Dr Bruce Thorley, Senior Medical Scientist and Head, WHO Regional Poliomyelitis Reference Laboratory, VIDRL, Melbourne discussed the VIDRL’s experience with rRT-PCR for ITD and VDPV screening. A total of 50 samples were tested by rRT-PCR ITD from 2010–2012. A total of 61 polioviruses were detected from these samples, including 41 single serotypes and nine mixtures. There were total of 18 PV1, (14 SL and four VDPV); 22 PV2, (14 SL and eight
VDPV); and 21 PV3,(19 SL and two VDPV). The samples were referred from Brunei Darussalam (two), Malaysia (10), Papua New Guinea (two) and the Philippines (36). The average ITD reporting time for Brunei Darussalam was two days (plus four days virus culture), while the average for Malaysia, Papua New Guinea and the Philippines was four days.

VIDRL has been accredited since December 2009 and has been using single-stage amplification procedure. The laboratory ensures that the same version of software is loaded on PCR machine and computers for analysis. The laboratory routinely performs nucleic acid extraction. It was emphasized that it is important that supervisor checks the results before reporting. There should be correct reagent addition and after addition the tube or the plate has to be spun down. The analysis of results was also presented using auto or manual baseline and threshold settings. In a typical amplification plot, the ABI7500 software automatically calculates baseline and threshold values based on the assumption that the data exhibit a typical amplification plot which has four distinct sections, namely, plateau, linear, exponential and baseline phases. The threshold is set correctly when in the exponential phase of the amplification curve. The threshold (Ct) should be checked in log and linear graph options since it is more difficult to assess Ct in log phase with variable amplification curves. The use of multicomponent plot assists in the assessment of the amplification plot. Caution with the low cycle thresholds (Cts) was emphasized because ABI software will report "undetermined" for Ct < cycle 4. Therefore, in-house nested pan EV threshold is set at ≥ Ct 6.

Dr Thorley also presented the results of the 2010 ITD and VDPV rRT-PCR, the 2011 ITD proficiency panel, and 2012 ITD sample, negative and positive controls. The data showed that there were lamp instability and power supply irregularities, missing data or program terminated by anti-virus software and Windows update. A need for the instrument maintenance on an annual basis was highlighted. After every Region of Interest (ROI) calibration, background calibration, optical calibration, dye calibration, and instrument verification must be performed. Successful calibration showed all wells have green circles. The lamp status should also be checked. A lamp bulb should be replaced when it is not functioning well and lamp usage is above 2000 hours. The laboratory staff should ensure that the current is stable and there is no indication of low current since this may indicate a potential failure of the lamp. Poliovirus sequencing protocol being used in VIDRL was provided.

2.1.6 Screening Sabin-like viruses for possible VDPVs

Mr Brian Emery discussed VDPVs and the screening method used to screen Sabin-like (SL) viruses. VDPVs have genetically mutated from the strain contained in the oral polio vaccine. It is based upon extent of VP1 nucleotide (nt) divergence from corresponding Sabin strain. Wild poliovirus has a 15% nt divergence, OPV-like has less than 1% nt divergence and VDPV has a 1%–15% nt divergence (and 1% demarcation arbitrary, consistent with one year replication or circulation). There are three types of VDPVs: (i) circulating vaccine-derived poliovirus (cVDPV), (ii) immunodeficiency-related vaccine-derived poliovirus (iVDPV), and (iii) ambiguous vaccine-derived poliovirus (aVDPV). There were several cVDPV outbreaks reported in the GPLN. A real-time PCR will be used for VDPV detection. The Sabin VDPV assays target a “key” antigenic site in VP1 such as: Sabin 1 (amino acid #99), Sabin 2 (amino acid #143), or the Sabin 3 (amino acids #285-291). The 3D gene is not a target anymore with the current assays.

Mr Emery also enumerated the laboratory routine procedures in setting up the assay from sample and reaction set-up to reporting of results. Real-time PCR worksheet for VDPV was
The use of the ABI 7500 machine and software 2.0 was demonstrated. In data analysis, VDPV assays only amplify the normal Sabin strains in the VP1 region and negative VP1 result indicates possible VDPV and should be sent for sequencing to identify the changes in VP1 regions. He also demonstrated ways to analyse and interpret the results of the assay.

2.1.7 ABI 7500 maintenance and calibration

Mr Emery discussed when and how to calibrate the ABI 7500 machine. The machine must be calibrated when installing the system in the following order: ROI, background, optical dye and instruments verification. The recommended period of calibration is every six months or as often as necessary, depending on instrument use and after replacing the lamp. It is very important that after every ROI, background calibration, optical calibration, dye calibration and instrument verification must be performed. The ROI maps the positions of the wells on the sample block so that the software can associate increases in fluorescence during a run with specific wells of the plate. Because the instrument uses a set of optic filters to distinguish the fluorescence emissions gathered during the runs, a calibration image of each individual filter to account for minor differences in the optical path is required. He also discussed troubleshooting on the condition of ROI, background calibration, optical calibration and spectral calibration. The lamp status should also be checked, and lamp bulb should be replaced when it is not functioning well and lamp usage is above 2000 hrs. The laboratory staff should ensure that the current is stable and no indication of low current since this may indicate a potential failure of the lamp.

2.1.8 Quality assurance and good laboratory practices

Dr Thorley discussed the quality assurance and good laboratory practice (GLP). VIDRL is a major public health facility which complies with ISO 15189:2009. GLP is necessary because the laboratory wanted to have confidence in providing timely, accurate, sensitive and appropriate laboratory results. Also, diagnostic and therapeutic laboratories work within a highly-regulated framework. GLP ensures high-quality and reliable test results, promotes the quality and validity of test data, and is underpinned by a quality system concerned with an organization's management. Quality control pertains to internal monitoring, parallel testing and maintaining competency, whereas quality assurance involves external monitoring and participation in appropriate proficiency programmes.

Quality system is defined as a structured and documented management system that describes the policies, objectives, principles, organizational authority, responsibilities, accountability and implementation plan of an organization for ensuring quality in its work processes, products (items), and services. There are four elements of a quality system: (i) a quality system manual that states what the organization does and how the quality system is put into practice, (ii) a defined organizational structure, (iii) good management practices, and (iv) good documentation. A document-controlled laboratory manual is essential. Standard operating procedures (SOPs) should be detailed, accurate, up-to-date and meet regulatory requirements. The laboratory should ensure an adequate number of trained staff is available for all procedures at all times and should maintain staff competency. Biosafety programmes should be in place and include staff immunizations, adequate training and supervision. A documentation trail of laboratory records is necessary to clearly indicate why, how and by whom the test was done, who supervised, what equipment was used, the results obtained, if problems were encountered and how they were addressed. Biological safety cabinets and good laboratory infrastructure are also essential for the safety of the staff. Traceability should be established, to
trail data from receipt of a specimen in the laboratory to reporting of the final result. Equipment records should be kept and regular equipment maintenance should be implemented.

Enrolment in annual proficiency panels is an accreditation requirement to ensure staff is trained, to verify methods and procedures, identify problems, assess performance, comparison with peers, and demonstrate competence of the laboratory staff. Laboratories within the WHO polio laboratory network undergo an annual accreditation site review using WHO polio accreditation checklist. Accreditation is a fact-finding mission, not a fault-finding mission. Internal audits are also important for reviewing internally a specific item across the institute, investigating whether section management/procedures conform to the quality system manual, key processes for system maintenance and improvement, and whether assistance with establishing institutional policy is necessary.

Regulatory requirements, including package-compliant protocols and mandatory training, are of significant importance in transporting infectious substances. The shipper needs to be qualified and re-certified every two years. Based on WHO Guidance on regulations for the transport of infectious substances 2011–2012, there are two infectious substance categories: category A, indicative list UN 2814 (an infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy human or animals; polio isolate is included), and category B, UN3373 Biological substance (an infectious substance which does not meet the criteria for inclusion in category A such as stool samples). An import permit is needed when sending polio isolates to the RRL.

2.1.9 Troubleshooting real-time PCR for poliovirus identification

Mr Brian Emery explained the troubleshooting real-time PCR poliovirus identification. He cited some problems and possible causes, which are listed in Table 1.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>All reactions negative, including positive control</td>
<td>Component missing, wrong thermocycler profile used or bad reagent</td>
</tr>
<tr>
<td>No Ct value with positive control; some sample reactions positive</td>
<td>Control RNA degraded or not added</td>
</tr>
<tr>
<td>Ct value with negative control positive</td>
<td>Reagent contaminated with virus or amplified DNA. Obtain new reagent control</td>
</tr>
<tr>
<td>Positive Ct values with one or more Sabin pairs, but corresponding Serotype pairs and/or PanPV are negative</td>
<td>Ensure that PCR was performed with 44°C annealing temperature. Ensure that ramp time for 44 °C to 60 °C step is approximately 45 seconds (i.e. 25% rate with ABI 7500)</td>
</tr>
<tr>
<td>Positive reaction (Ct value) with PanPV primers but all serotype pairs are negative</td>
<td>Suspect low virus titers and rerun with RD TC</td>
</tr>
</tbody>
</table>
Mr Emery demonstrated how to manipulate the ABI 7500 software to solve some possible problems with the real-time PCR results. He also mentioned that the wells can be damaged, which can lead to no assay results. The real-time PCR kits stored at -20°C for four years have been tested and found to be stable, and all kits from 2012 will have four years expiration date when stored at -20°C. In conclusion, it is necessary to check the following: (1) run method time and temperature are correct, (2) no passive reference dye is selected, (3) target dye assignment, (4) ramp speed (anneal to extension) is always 25%, (5) automatic versus manual baseline reset, (6) for dual stage real-time PCR, check if each stage is correct, (7) only collect fluorescence data at anneal step, and (8) calibrations are current and passed Rnase P test.

2.1.10 Proficiency test panels for poliovirus ITD and VDPV screening by rRT-PCR

Mr Brian Emery facilitated the discussion of the proficiency test (PT) panels. The objective of PT is to test the proficiency of the laboratories. Molecular PT panels consist of unknown lyophilized non-infectious RNA which should be reconstituted with 50ul of water supplied (re-suspend completely), kept at room temperature for 20–30 minutes, and placed inside the freezer overnight. After this process, the sample is ready for testing. Real-time PT panels should be tested like normal/routine samples. Insert reporting should be followed. For real-time PCR VDPV PT panels, separate panels of Sabin-related unknowns (possible VDPVs and non-VDPVs) are provided. These unknowns should be tested with real-time VDPV kits only. The Sabin's that were detected in the ITD PT panel should not be tested for VDPV screening. The scores of the real-time PCR ITD and VDPV PT panels are based on the 50% of scores on results and 50% score on the interpretation of the results. The results should be reported within seven days. Five per cent from the total score will be deducted for every week that the report is late.

2.1.11 Implementation planning

Dr Bruce Thorley mentioned that the laboratory needs to assess their equipment, space, reagents, supplies, regular and back-up personnel, and standard operating procedures (SOPs) to implement the real-time PCR ITD and VDPV assays. The laboratory should anticipate workflow changes like discontinuation of electrophoresis and ELISA, and changes in data entry. To attain proficiency, at least three test runs (10 samples per experiment, all primer sets) should be done. It is recommended to use stored isolates of known PV serotype and intratype in both ITD and VDPV rRT-PCR. To gain proficiency, select from among isolates that have been sequenced already: wild virus (if available), SL viruses that were sequenced because of discordant ITD, NIBSC standard Sabin strains (to represent SL monotype), make-up samples to represent mixtures using NIBSC strains, and known VDPV (if available).

Retrospective testing will be performed on Sabin-like isolates to screen for VDPVs and only VDPV primers should be used. A list of Sabin-positive cases from 2004 to 2012 is needed. To maximize efficiency and minimize reagent needs, setting-up in batches and one isolate per specimen (unless isolate has a PV mixture) is recommended. Results should be entered on a Microsoft Excel spreadsheet (cumulative) and sent to WHO and US CDC for review and feedback.

Prospective parallel testing of newly-identified isolates will be done following the flowchart for new algorithm in terms of workflow. Two methods will be run (traditional PCR, rRT-PCR), and two VDPV screening methods will be run (ELISA and rRT-PCR). There is no need for neutralization test before rRT-PCR for PV mixtures, and rRT-PCR will be done on all
identified SL viruses. However, a neutralization test must be done before ELISA for PV mixtures. Results will be sent at the end of each run on a Microsoft Excel spreadsheet.

Timeline for implementation of rRT-PCR ITD and VDPV screening assays was scheduled as follows: (i) phase 1: attaining proficiency by the end of January 2013; (ii) retrospective VDPV screening by January to February 2013; and (iii) prospective testing beginning January 2013, likely to continue until the end March 2013. Real-time PCR proficiency test panel will be evaluated.

2.1.12 Biosafety training

Dr Bruce Thorley discussed biorisk management. A biorisk management training course is important because it promotes biosafety and biosecurity awareness in the GPLN. A training course would also promote professional development, inform staff of recent developments, improve existing practices and instigate change to management practices. Laboratory biorisk management is a system or process to control safety and security risk associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities, and is based on CWA 15793:2011 standards defined by the European committee for standardization CEN Workshop Agreement in 2011. Biorisk encompasses risks from the biosafety and laboratory security. Biorisk management is equivalent to the assessment, mitigation and performance (AMP) model.

The first stage in the biorisk management process is to identify all hazards and threats that are relevant for biorisk. A hazard is a source or object that can cause harm and it is not a risk without a specific environment or situation. Risk is the likelihood of an event with a hazard that has consequences. Likelihood is the probability of an adverse event occurring, while consequence refers to the severity of the event. The use of a risk matrix to identify the likelihood and consequence categories to illustrate those falling into high, moderate and low risk zones was presented. The participants were divided into two groups to conduct exercises on biorisk assessment using the BioRisk Assessment Model (BioRAM). BioRAM lite is the version that was created for this training course. It was simplified, and many critical questions and details were omitted. It also has limitations and should not be used to make actual laboratory safety/security decisions.

In conducting risk assessments, due considerations should be made of the inherent risks from the biological agents and toxins (e.g. pathogen risk grouping descriptions, such as morbidity and mortality, treatment and prevention, routes of transmission, communicability, agent stability), as well as how risks change based on the laboratory infrastructure and use of biological agents in the laboratory. Additional information needed for risk assessment includes people, environment, mitigation measures and financial aspects. The results of the assessments should be used to determine and implement the risk mitigation measures to reduce risk to acceptable levels. Risk acceptance will depend on the owner of the risk, and may be risk averse or risk tolerant. Mitigation control measures should consider hierarchy of hazard controls.

The process of setting and reviewing objectives and implementing programmes to achieve them provides a mechanism for the organization to continually improve its biorisk management system and to improve its biorisk performance. Performance includes control (processes, procedures, structures and responsibilities to manage biorisk), assurance (systematic process of checking the system through audits and inspections), and improvement (setting and achieving biorisk goals based on internal and external feedback). The group was given an exercise to
identify problems and the consequences of the risk involved in assessment, mitigation and performance.

The laboratory biorisk management standard is based on CWA 15793:2011, a management system approach, and is consistent with other international standards such as ISO 9001/14001 and OHSAS 18001. It is a performance-based, voluntary and Plan-Do-Check-Act (PDCA) based systematic approach. Another exercise activity was given to the group to develop recommendations for the biorisk cases using the table of contents of the CWA 15793:2011.

2.2 Country Reports

2.2.1 Hong Kong (China)

Ms Amanda To Pui Chi from the Public Health Laboratory Centre presented the country report of Hong Kong (China). Poliomyelitis is a notifiable disease since 1948. In 1963, polio vaccination was introduced. In 1996, the National Committee for Certification of Wild Poliovirus Eradication was established, followed by introduction of AFP surveillance and accreditation of the national polio laboratory in 1997.

The last case of wild poliovirus was reported in 1983. In 2007, IPV replaced the OPV. She discussed the classification of AFP cases from 2010–2012. Among AFP cases reported and classified from 2010–2012, there has been no polio-compatible case found. There was a decrease in the number of non-polio AFP cases per 100 000 people below 15 years old, from 1.8 to 1.2 in 2011. However, the performance indicators of the surveillance are above the target. The laboratory has consistently obtained 100% scores in WHO PT and timeliness of reporting was also 100%.

The immunization coverage for polio vaccines based on administrative data from 2007–2011 ranges from 58.99% to 99.70% among children from the ages of two months to 11 years old. Cell sensitivity results of RD and L20B cell lines were within acceptable ranges. There was no poliovirus detected among the 31 AFP samples received in 2010, the 35 samples in 2011, and the 34 samples in 2012. There were 2523 non-AFP stool samples submitted in 2010, 1334 samples in 2011, and 1301 samples in 2012. No poliovirus isolate was found from the non AFP stool samples in 2010 and 2012, but there was one poliovirus isolate in 2011. There was one poliovirus isolate in 2010 and three poliovirus isolates in 2011 from non-AFP samples other than stools. ITD results showed that all poliovirus isolates from 2010–2011 were Sabin type 2.

The laboratory also participated in the ITD PT using conventional PCR from 2010–2012 and obtained a100% score. She also discussed the testing algorithm for hand-foot-mouth-disease (HFMD). She also mentioned the challenges in establishing real-time RT-PCR for ITD and VDPV detection as well as establishing sequencing of mixed poliovirus.

2.2.2 New Zealand

Ms. Judy Bocacao from the Institute of Environmental Science and Research (ESR) discussed the country report for New Zealand. In the country with a population of approximately 4.4 million people, the expected AFP cases are eight to nine per year. The Ministry of Health is responsible overall for polio surveillance, laboratory and immunization activities. Paediatricians are required to report AFP cases to New Zealand Paediatric Surveillance Unit (NZPSU) immediately. All paediatricians (163) are required to provide monthly report to NZPSU. ESR
Clinical Virology Laboratory is the only WHO-accredited laboratory to conduct tests for all AFP cases.

There were four AFP cases each in 2010 and 2011 and six in 2012. The total numbers of AFP stool samples received in 2010, 2011 and 2012 were eight, six and 14, respectively. No poliovirus was detected from the AFP samples. Three non-polio enteroviruses (NPEV) were detected in 2010 and 2011: one EV71 and one EV74 were detected in 2010 and one EV71 was detected in 2011. Aside from AFP stool samples, the laboratory also tests clinical stool samples. The NPEV rate from these clinical stool samples was 37.5% in 2010, 36% in 2011 and 11.5% in 2012 (as of October).

Cell sensitivity testing of RD cell line showed that the results were within the acceptable range in all three polio serotypes. L20B cell line showed that the results of the two serotypes, poliovirus 1(PV1) and PV2, were within the acceptable range but PV3 titer started to decline. Repeat testing is recommended for this particular serotype, however, if the results remain the same, L20B cell line should be replaced and a new batch should be requested from VIDRL. The laboratory also participated in the ITD PT using conventional PCR from 2010–2012 and consistently obtained 100% scores.

To supplement the AFP surveillance, the National Enterovirus Surveillance System was established to identify circulation patterns of enterovirus serotypes and predominant strains and, describe clinical diseases associated with circulating serotypes, and detect enterovirus outbreaks to assist public health intervention. The highest incidence of EV71 was recorded in 2007 from 15 cases. In 2010 and 2011, the reported cases of EV71 were eight and nine respectively. Enterovirus 68 (EV68) was identified for the first time in 2010 in New Zealand. Out of the 15 samples isolated with EV68, 11 (73%) were from children less than two years of age. Majority came from South Auckland (87% or 13 out of 15) and a small proportion from Waikato (13% or 2 out of 15). In addition, enterovirus 74 was identified in New Zealand for the first time in 2011 from a faecal sample of a two-year old boy with AFP from Auckland. Enterovirus 109 was also newly identified in 2011 and was isolated from a nasopharyngeal aspirate from a one-year-old male child with bronchiolitis. The laboratory acquired a new machine, iPrep™ Purification Instrument, an automated purification of nucleic acids using magnetic beads.

2.2.3 The Philippines

Dr Lea Necitas Apostol from Research Institute for Tropical Medicine (RITM) presented the country report of the Philippines. RITM is the research arm of the Department of Health and provides laboratory support for the Department of Health for surveillance and outbreak investigation. In RITM, there are four WHO-accredited national laboratories: the national polio laboratory, national measles laboratory, national Japanese encephalitis (JE) laboratory, and national rotavirus laboratory.

The performance indicators of the AFP surveillance were presented. Out of 17 regions (regional surveillance units) in the country, only seven regions submitted two adequate stool samples collected within 14 days of onset. Around 50% of the regions submitted stool samples within four days, others submitted after more than four days, some submitted stool samples after more than 30 days. Majority of the stool samples were received in good condition. The laboratory performance indicators in 2011–2012 showed that the timeliness of reporting was around 95%. The accuracy of PV detection and identification among all poliovirus isolates was
100% in 2011 and 2012. The scores from the WHO PT in 2011 and 2012 were 100%, and the score from the annual on-site review was 97% in 2011.

She presented the results of her scientific paper on the occurrence, diversity and pattern of circulation of NPEV serotypes implicated in AFP surveillance. The results showed that there was a very high degree of enterovirus diversity (recent/new serotype, rare serotype), that the isolation trend is consistent with all tropical countries (human enterovirus (HEV)-B, HEV-C, HEV-A), and that multiple patterns were observed (endemic, cyclic, epochal). However, some limitations were observed, including overall data may be underrepresented, overrepresentation of neurotropic NPEV types and virus isolation (underdetected for some coxsackieviruses (CVs) versus favoured serotypes).

Cell sensitivity tests of RD and L20B cell lines showed that the results are within acceptable ranges. The laboratory has prepared a quarterly polio bulletin to facilitate data feedback from the field and laboratory surveillance performance to stakeholders (WHO, National Epidemiology Center of the Department of Health) and the regional surveillance sites. Also, staff from the laboratory attend the quarterly expert panel review meeting for AFP case classification.

The national polio laboratory also started enterovirus surveillance and testing for the hand-foot-mouth-disease (HFMD) in June 2012 for the confirmation of enterovirus 71 (EV71). Type of specimens collected were: swabs (oropharyngeal/throat, rectal, vesicular), CSF and stool. The samples were tested using PCR, pan-EV nested PCR for screening and EV71-specific RT-PCR for confirmatory. There is no specific surveillance for EV71 being implemented based on little knowledge about its prevalence, diversity or spectrum of disease locally. AFP surveillance was implemented to detect poliovirus (PV) and other enteroviruses. From 1992 to 2008, there were eight EV71 isolated and detected and, similar to the situation in other countries, EV71 infection has been associated with a subset of AFP cases. This HFMD surveillance provides the first epidemiologic and virologic survey of EV71 in the country. Subgenogroup C2 was identified, which has a wide circulation both in tropical and temperate countries.

2.2.4 Republic of Korea

Mr Sang-gu Yeo from Korea Centers for Disease Control and Prevention reported on the national polio laboratory (NPL) in the Republic of Korea. The estimated total population of the country is about 50 million people. Polio vaccination has been carried out by national vaccination programme since the 1960s. In 1983, the last wild poliomyelitis case was reported. Inactivated polio vaccine (IPV) has been implemented in 2005 and about 95% of people have been immunized. The non-polio AFP rates in 2010 and 2012 were 0.88% and 0.97% respectively.

In the AFP surveillance, there are 50 participating paediatric neurology hospitals. An AFP enhancement research project is in progress through the Catholic University of Korea. The laboratory diagnosis of enteroviruses is done using real-time RT_PCR (Pan-EV (5’NCR)/EV71-specific (VP1)). Serotyping and genotyping are done on positive samples. The cell sensitivity test for RD and L20B cell lines showed that the results were within acceptable ranges. The total AFP cases reported was 70 in 2010, 31 cases in 2011 and 75 cases in 2012. The percentage of AFP cases with adequate stool samples in 2011 was 90.6% and 98.6% in 2012. These AFP cases were reported with different medical presentation. In 2012, there were 25 reported cases with
The enterovirus laboratory surveillance network (ELSN), established in 2006, is composed of the NPL, 38 surveillance hospitals and seven public health regional institutes. Nearly 2000 enterovirus cases are reported annually by the ELSN with most cases reported from Seoul, Busan, Kyeonggi, and Gwangju regions. In 2010, there was a slight increase of the frequency of enterovirus detected and notably, all polioviruses detected were Sabin strain. There were major outbreaks of EV71 in 2009 and 2012 which resulted in the death of two patients in 2009 and one patient in 2012. These were also associated with the outbreak of neurologically-complicated EV71 infection during 2010–2012. Genotype C4a was detected based on the phylogenetic analysis of the VP1 gene of EV71 circulating in the Republic of Korea.

Development of EV71 vaccines and serosurvey of polio vaccine were discussed. Vero cell line is used for the development of EV71 vaccine. There are several candidate vaccines being tested. For humoral immunity, cross-neutralizing antibody test is performed and for cellular immunity, intracellular cytokine staining (ICS) by Fluorescence Assisted Cell Sorting (FACS) assay is done. To determine the effectiveness of poliovirus vaccination, a total of 720 cases within the range of six to 90 years old were included. The results showed that the seroprevalence rates were 70% for PV1, 85% for PV2 and 55% for PV3.

The laboratory has a web-based enterovirus reporting system, reports the data via e-mail to the WHO Regional Office for the Western Pacific, and shares the data with national expanded programme on immunization (EPI) or surveillance. There is a monthly training of NPL members on biosafety in the laboratory. A biosafety manual is available and a biosafety officer has also been appointed.

2.2.5 Viet Nam (Hanoi)

Ms Nguyen Thi Thu Trang from National Institute of Hygiene and Epidemiology reported the surveillance and diagnostic activities of the national polio reference laboratory in Northern Viet Nam. The workload of laboratory includes AFP surveillance, HFMD surveillance, and rotavirus surveillance. It works closely with national expanded programme on immunization (EPI) to build a good surveillance system for polio and rotavirus.

There were 191 AFP cases and 194 AFP cases in 2010 and 2011, respectively. In 2012, 194 AFP cases were also reported. Among the reported cases, there were two (PV2), one (PV3), and two (PV1) laboratory-confirmed polio in 2010, 2011, and 2012, respectively. There were 38 NPEV cases detected in 2012 and 16 NPEV cases detected in 2011.

In Northern Viet Nam, it was noted that the coverage of polio vaccination in children younger than one year old decreased from 99.9% in 2010 to 82.6% in 2012. Timeliness of reporting for the virus isolation using the new algorithm (within 14 days) in 2012 was 96.23%. The cell sensitivity testing results of RD and L20B cell lines showed good results, the titer of the three polio serotypes were within the acceptable range.

HFMD surveillance in Northern Viet Nam is conducted regularly through sentinel-based reporting units to determine the causative agent of the HFMD outbreak. The types of clinical samples received are: throat swab, vesicle fluid, and faeces for the diagnosis. Methods being used to diagnose HFMD are semi-nested RT-PCR/sequencing, and virus isolation (using RD,
There were 262 EV71 detected from HFMD cases in 2011, while 223 EV71 were detected in 2012.

2.2.6 Viet Nam (Ho Chi Minh City)

Mr Nguyen Trung Kien of Pasteur Institute presented the updates from the Laboratory of Enteroviruses which is responsible for surveillance of enteroviruses in Southern Viet Nam. The laboratory has been a member of the WHO Laboratory Network for the Poliomyelitis Eradication Programme in the Western Pacific Region since 1992. The number of AFP cases reported increased from 109 in 2010 to 263 in 2012. Polioviruses were detected from 2010–2012. There were two PV3 Sabin-like isolated in 2010, one each (two) PV1 SL, PV2 SL in 2011, and five (1 PV1 SL, 1 PV2 SL, 1 PV3 SL, 2 type 2-VDPV) in 2012. There were six EV71 detected in 2011 and 22 EV71 confirmed in 2012. The two cases with L20B positive were sent to the National Institute of Infectious Diseases (NIID) in Japan in April and May in 2012 and the test results showed that the two cases were type 2-VDPV. Collection of samples from contacts (where patients live) was performed and test results showed all 29 samples were negative. The laboratory received a new batch of RD and L20B cell lines from VIDRL on 23 February 2012. Cell sensitivity tests were performed with all three poliovirus serotypes midway through their use of 15 passages. The results of the cell sensitivity were within the acceptable range. The results were reported to regional laboratory coordinator within 48 hours.

2.3 Practical sessions

The hands-on training session was conducted in the lecture room and laboratory of RITM. It included five days of lectures and practical sessions. For the group activities, country participants were divided into four teams. All teams shared one ABI 7500 machine for rRT-PCR:

- Team 1: Philippines (two participants)
- Team 2: Viet Nam (two participants)
- Team 3: Hong Kong (China) (one participant) and Republic of Korea (one participant)
- Team 4: New Zealand (one participant)

On the first day, the practical session started with setting-up the real-time ITD PCR assay and a programming exercise on ABI 7500 real-time PCR machine. The participants were supervised by the advisers, from preparing sample assays until participants were ready to use the ABI 7500 machine. The practical session in the laboratory was followed by discussion on data analysis of ABI machine output. Working in pairs, participants performed and analysed rRT-PCR reactions. All primer sets used degenerate PCR conditions. For the first experiment, team 1 received PV1-non-Sabin-like (NSL) as unknown A, and PV1 Sabin-like (SL) and PV3-SL as unknown B. Team 2 received PV3-NSL as unknown A and PV2 SL and PV3 SL as unknown B. Team 3 had PV2-NSL and PV1-SL and PV2-SL as unknown A and B respectively. Team 4 had PV1 NSL and PV2 NSL for unknown A and PV1 SL, PV2 SL and PV3-SL for unknown B. ITD assay results interpretation was then discussed such as if the result is possible polio, it should be referred for sequencing and if the result shows PV1-SL, VDPV1 assay should be run.
The experiment on the second day involved setting-up or loading VDPV ITD real-time PCR assay. There was an introduction to laboratory set-up, preparation of worksheets, calculations of reagents before bench work. The VDPV assays used normal, non-degenerate, RT-PCR profiles. For the second experiment, team 1 received S1 VDPV and S3 VDPV as unknown B. Team 2 received S2 VDPV and S3 VDPV as unknown B. Team 3 had S1 VDPV and S2 VDPV as unknown B and team 4 had S1 VDPV, S2 VDPV, and S3 VDPV for unknown B. The interpretation of VDPV results was also discussed. If VP1 is positive, SL should be reported and if VP1 is negative, NSL should be reported and should be referred for sequencing.

On the third day, the third and fourth experiments were conducted. The third experiment involved setting-up or loading unknown samples for rRT-PCR ITD assay. The participants used all primer sets and appropriate controls. Team 1 received PV1SL, PV2 NSL and PV3 SL as unknown 1 and PV1 SL, PV2 SL and PV3 SL as unknown 2. Team 2 received PV2 SL and PV3 NSL as unknown 1 and PV1 SL, PV2 NSL and PV3 SL for unknown 2. Team 3 had PV2 NSL as unknown 1 and PV2 SL, PV2 SL and PV3 SL as unknown 2. Team 4 had PV1NSL and PV2 NSL for unknown 1 and PV2 SL and PV3 SL for unknown 2. The fourth experiment involved setting-up VDPV, ITD real-time assay from the previous experiment unknown. Participants only tested the SL positive samples from the third experiment using the respective primer sets and appropriate controls. The experiments were followed by discussion on troubleshooting of assays and results.

On the fourth day, continuation of the fourth experiment was done with VDPV test run, followed by a discussion and presentation of results. Dr Bruce Thorley and Dr Youngmee Jee gave presentations on data management, ITD data reporting, and real-time PCR implementation planning and discussion.

On the fifth day of the training, biorisk management training, discussions and third to fifth group activities continued. The third activity of biorisk management training sought the performance measures needed to be incorporated for each mitigation measure implemented. The fourth activity focused on identifying problems in assessment, mitigation, and performance. Lastly, the fifth activity used table of contents of the CWA15793 to develop recommendations for the biorisk cases. The Western Pacific Region implementation plan of rRT-PCR was also discussed by Dr Youngmee Jee.

3. CONCLUSIONS

3.1 General

The three main objectives of the training were achieved during the five-day training workshop through intensive hands-on practical sessions, lectures, and group activities. At the end of the workshop, the technical laboratory capacity and knowledge of the participants were enhanced. The participants were able to learn the new real-time PCR technique for rapid
detection and characterization of polioviruses. Also, hands-on practice on real-time techniques for ITD of polioviruses and VDPV screening using real-time PCR platform were fully learnt by participants.

3.2 Workshop evaluation

All of the participants gave positive feedback about the entire workshop. They expressed that they obtained enough information and experience from the workshop to enable them to begin using the methods in their respective laboratories. Among the most useful topics learnt were hands-on experience with rocket rRT-PCR, analysis and troubleshooting of the data, and biorisk management training.

Participants were encouraged to contact each other, the facilitators and the WHO Regional Office for the Western Pacific to follow up on practical issues such as quality assurance. The schedule was followed efficiently and every set of tasks for the day were completed on time.

The staff in RITM gave their full support and assisted the participants in their laboratory activities and during the lecture sessions. The duration of each presentation allowed adequate time for further discussion on theoretical, technical, and practical issues.

3.3 Outcomes of training

The participants from most polio laboratories in the Region were trained to perform the new real-time PCR technique for rapid detection and characterization of polioviruses using dual stage real-time PCR techniques for ITD of polioviruses and VDPV screening developed by US CDC. This training contributes to reducing the time needed for ITD and the frequency of shipping of virus isolates from national laboratories to the regional reference laboratories or global specialized laboratory for ITD and sequencing. As a result, ITD results of polioviruses will be available earlier for appropriate programme actions.

It is expected that most polio network laboratories in this Region will conduct real-time PCR for ITD and VDPV screening by mid-2013. After the successful completion of PT, the participating laboratories will be upgraded to polio ITD laboratories. The laboratories will be assessed for ITD function during the next WHO annual accreditation visits.

At the end of the workshop, the ITD and VDPV screening kits were distributed to participants. Participants were requested to report results of implementation steps to US CDC, VIDRL and the WHO Regional Office for the Western Pacific.

3.4 Follow up to the workshop

The country implementation plan for rRT-PCR should be sent by 8 January 2012 to the Regional Office for the Western Pacific. Both the revision of SOP and development of worksheets are due on 30 January 2013. All participating laboratories should submit results of each step to the Regional Office for the Western Pacific, US CDC and VIDRL. Final step of PT panel samples will be sent from US CDC to the participating laboratories after successful completion of steps one and two. The results of step one should be sent before 31 January 2013 within 48 hours after test completion. Retrospective sample testing of step two should be sent by 28 February 2013. Prospective sample testing for step three can be performed with step 2 once step 1 is successfully completed.
Until the participating laboratories complete the four implementation steps, all polio isolates should be sent to the designated WHO regional reference or global specialized laboratories, US CDC, VIDRL and WHO will review the outcomes of each step to ensure successful implementation of the techniques. After the successful completion of implementation steps, polio laboratories which participated in this workshop will be upgraded to polio ITD laboratories.