Fifth Meeting on Vaccine-Preventable Diseases Laboratory Networks and Poliovirus Biorisk Management Training in the Western Pacific Region

25-30 May 2015
Manila, Philippines
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REPORT

FIFTH MEETING ON VACCINE-PREVENTABLE DISEASES LABORATORY NETWORKS AND POLIOVIRUS BIORISK MANAGEMENT TRAINING IN THE WESTERN PACIFIC REGION

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REGIONAL OFFICE FOR THE WESTERN PACIFIC

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NOTE

The views expressed in this report are those of the participants of the Fifth Meeting on Vaccine-Preventable Diseases Laboratory Networks and Poliovirus Biorisk Management Training in the Western Pacific Region and do not necessarily reflect the policies of the conveners.

This report has been prepared by the World Health Organization Regional Office for the Western Pacific for Member States in the Region and for those who participated in the Fifth Meeting on Vaccine-Preventable Diseases Laboratory Networks and Poliovirus Biorisk Management Training in the Western Pacific Region in Manila, Philippines from 25 to 30 May 2015.
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Keywords:

Poliovirus vaccines /Poliomyelitis-prevention and control/Vaccines/Laboratories/Measles/Rubella
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AFP</td>
<td>acute flaccid paralysis</td>
</tr>
<tr>
<td>aVDPV</td>
<td>ambiguous vaccine-derived poliovirus</td>
</tr>
<tr>
<td>bOPV</td>
<td>bivalent oral polio vaccine</td>
</tr>
<tr>
<td>CAP</td>
<td>College of American Pathologist</td>
</tr>
<tr>
<td>CVA6</td>
<td>coxsackievirus A6</td>
</tr>
<tr>
<td>CVA16</td>
<td>coxsackievirus A16</td>
</tr>
<tr>
<td>CRS</td>
<td>congenital rubella syndrome</td>
</tr>
<tr>
<td>DBS</td>
<td>dried blood spot</td>
</tr>
<tr>
<td>DTaP-IPV</td>
<td>diphtheria, tetanus, pertussis and polio</td>
</tr>
<tr>
<td>iVDPV</td>
<td>immunodeficiency-related vaccine-derived poliovirus</td>
</tr>
<tr>
<td>EV71</td>
<td>enterovirus</td>
</tr>
<tr>
<td>EPI</td>
<td>Expanded Programme on Immunization</td>
</tr>
<tr>
<td>EQA</td>
<td>external quality assessment</td>
</tr>
<tr>
<td>ES</td>
<td>environmental surveillance</td>
</tr>
<tr>
<td>FTA</td>
<td>Fast Technology Analysis</td>
</tr>
<tr>
<td>GAP III</td>
<td>Global Action Plan</td>
</tr>
<tr>
<td>Gavi</td>
<td>Global Alliance for Vaccines and Immunisation</td>
</tr>
<tr>
<td>GPLN</td>
<td>Global Polio Laboratory Network</td>
</tr>
<tr>
<td>GPLNMS</td>
<td>Global Polio Laboratory Network Management System</td>
</tr>
<tr>
<td>HFMD</td>
<td>hand-foot-mouth disease</td>
</tr>
<tr>
<td>HPeV</td>
<td>human parechovirus</td>
</tr>
<tr>
<td>HPA</td>
<td>Health Protection Agency</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>immunoglobulin M</td>
</tr>
<tr>
<td>IHC</td>
<td>in-house control</td>
</tr>
<tr>
<td>IMR</td>
<td>Institute for Medical Research</td>
</tr>
<tr>
<td>IPV</td>
<td>inactivated poliovirus vaccine</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>ITD</td>
<td>intratypic differentiation</td>
</tr>
<tr>
<td>JICA</td>
<td>Japan International Cooperation Agency</td>
</tr>
<tr>
<td>KCDC</td>
<td>Korea Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>LabNet</td>
<td>Laboratory Network</td>
</tr>
<tr>
<td>L20B</td>
<td>a mouse cell line (L-cells), genetically engineered to express the human poliovirus receptor</td>
</tr>
<tr>
<td>MCV</td>
<td>measles containing vaccine</td>
</tr>
<tr>
<td>MeaNS</td>
<td>Measles Nucleotide Surveillance</td>
</tr>
<tr>
<td>MMR</td>
<td>measles mumps and rubella</td>
</tr>
<tr>
<td>MRSRS</td>
<td>Measles-Rubella Surveillance Reporting System</td>
</tr>
<tr>
<td>NL</td>
<td>national laboratory</td>
</tr>
<tr>
<td>NIBSC</td>
<td>National Institute for Biological Standards and Control</td>
</tr>
<tr>
<td>NIID</td>
<td>National Institute of Infectious Diseases</td>
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<tr>
<td>NIHE</td>
<td>National Institute of Hygiene and Epidemiology</td>
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<tr>
<td>NPEV</td>
<td>non-polio enterovirus</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>OPV</td>
<td>oral polio vaccine</td>
</tr>
<tr>
<td>PASRS</td>
<td>Polio AFP Surveillance and Reporting System</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PT</td>
<td>proficiency test</td>
</tr>
<tr>
<td>PV</td>
<td>poliovirus</td>
</tr>
<tr>
<td>QA</td>
<td>quality assurance</td>
</tr>
<tr>
<td>RCV</td>
<td>rubella containing vaccine</td>
</tr>
<tr>
<td>RD</td>
<td>human rhabdomyosarcoma</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>real-time reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>RITM</td>
<td>Research Institute for Tropical Medicine</td>
</tr>
<tr>
<td>RLC</td>
<td>regional laboratory coordinator</td>
</tr>
<tr>
<td>RubeNS</td>
<td>Rubella Nucleotide Surveillance</td>
</tr>
<tr>
<td>tOPV</td>
<td>trivalent oral polio vaccine</td>
</tr>
<tr>
<td>US CDC</td>
<td>United States Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>VDPV</td>
<td>vaccine-derived polio viruses</td>
</tr>
<tr>
<td>VIDRL</td>
<td>Victorian Infectious Diseases Reference Laboratory</td>
</tr>
<tr>
<td>VP1</td>
<td>viral capsid protein</td>
</tr>
<tr>
<td>WPV</td>
<td>wild poliovirus</td>
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</table>
SUMMARY

The Fifth Meeting on Vaccine-Preventable Diseases Laboratory Networks and Poliovirus Biorisk Management Training in the Western Pacific Region was held in Manila, Philippines from 25 to 30 May 2015 to review the performance and identify the challenges of the polio and measles/rubella network laboratories in the Region and to hold a four-day training to implement biorisk management for laboratories holding wild and oral polio vaccine (OPV)/Sabin poliovirus materials.

The Laboratory Networks (LabNet) meeting reviewed ways to strengthen the performance of network laboratories and also to monitor implementation of recommendations from the fourth vaccine-preventable diseases (VPD) laboratory networks meeting in 2013. The meeting also provided an opportunity to discuss ways to improve the quality and timeliness of laboratory testing among network laboratories including the subnational laboratories in China.

1. INTRODUCTION

1.1 Meeting organization

Seventy participants from network laboratories, advisers, observers and WHO staff attended the meeting, including 44 representatives from 16 countries (12 polio network laboratories and 18 measles/rubella network laboratories). The list of participants is available at Annex 1.

The meeting was organized in three sessions over six days to cover poliomyelitis (25–26 May), measles and rubella (27–28 May) and biorisk management training (27–30 May). The meeting programme is available at Annex 2.

1.2 Meeting objectives

The objectives of the meeting were:

1) to discuss and review performance and the implementation status of new requirements of the poliomyelitis network laboratories especially for intratypic differentiation (ITD) laboratories in the Region;

2) to identify challenges and define/develop plans for the expanding roles of the polio network laboratories to maintain polio-free status in the Region in line with Polio Eradication and Endgame Strategic Plan 2013–2018;

3) to review the progress and identify gaps/challenges of the measles and rubella network laboratories to support measles and rubella elimination and provide evidence to verify measles elimination; and

4) to develop plans to strengthen molecular detection capacity and ensure quality performance of network laboratories.

2. PROCEEDINGS

2.1 Polio LabNet

2.1.1 Polio endgame strategy: regional update on the polio eradication initiative and next steps
The WHO Regional Office for the Western Pacific detected its last indigenous wild poliovirus type 1 (WPV1) in 1997 and last imported wild poliovirus (WPV) in 2011. The last circulating vaccine-derived poliovirus (cVDPV) was detected in China in 2012. The non-polio acute flaccid paralysis (AFP) rate in the Region from 2013 to 2015 (week 18) exceeds one for all countries with the exception of Papua New Guinea and the Philippines. Polio risk assessment was carried out in 2014 and all countries were classified as low except China as medium and Cambodia, Papua New Guinea and the Philippines as high risk. Part of the polio endgame strategy is strengthening immunization systems and withdrawing OPV.

In 2015 the World Health Assembly adopted a resolution that recommended all countries switch from trivalent OPV (tOPV) to bivalent OPV (bOPV) in April 2016 to eliminate the risk of type 2 cVDPV. Seventeen countries (including 10 Pacific island countries) in the Region use OPV and these are planned to switch to bOPV + inactivated poliovirus vaccine (IPV). Malaysia and Singapore use a sequential schedule and will switch to all IPV. These countries will then be monitored to ensure all tOPV is withdrawn.

2.1.2 Global update

The last WPV2 was detected in 1999 and the latest WPV3 was found in 2012 (Nigeria and Pakistan). In 2015, WPV1 has been found only in Afghanistan and Pakistan. This is also the first time that WPV has not been seen in the African region for more than a six-month period. Nigeria has reported a decrease in the number of children with zero dose in non-polio AFP cases in the usual reservoir state of Kano. Nigeria is carrying out environmental surveillance (ES) in 11 areas and all have been negative for WPV for more than 12 months although one cVDPV2 was detected in Kaduna in March 2015. cVDPV2 viruses have also been detected in Pakistan and Somalia in 2015 which could impact on whether the switch to bOPV goes ahead as planned.

The performance of the Global Polio Laboratory Network (GPLN) continues to operate at a very high level with 98% of the 146 laboratories fully accredited. Three key projects are underway to improve sensitivity for detecting polioviruses; 1) improved diagnostic tools – real-time polymerase chain reaction (RT-PCR) assays with a more sensitive ITD version 4.0; direct detection of poliovirus from stools using magnetic beads; grab-bag sampling device for ES; 2) strengthening and extending ES to priority countries and areas; and 3) enhancing biosafety and biosecurity through a biorisk training programme and training to implement and monitor Global Action Plan (GAP III) introduction. Development of a web-based archive of data and indicators is underway to allow real-time access to all performance data. Challenges faced by the GPLN include: less funding, staff attrition, laboratory workload, a false sense that the job is done, workload of regional laboratory coordinators (RLC) and concern for the vacant RLC position in the WHO Regional Office for the Western Pacific for more than one year.

2.1.3 Polio laboratory network in the Western Pacific Region

The Western Pacific Region's Polio LabNet consists of 43 laboratories, 33 of which perform ITD. Four laboratories are performing environmental surveillance (ES) (Australia, China, Japan and Malaysia) and all are also performing enterovirus surveillance, including hand-foot- mouth disease (HFMD) and AFP. The Philippines is considering introducing ES.

The last cVDPV detected in the Region was in China in 2012. Small numbers of ambiguous vaccine-derived poliovirus (aVDPVs) and immunodeficiency-related vaccine-derived poliovirus (iVDPVs) were detected in China each year from 2012 to 2015 and an aVDPV was found in the Philippines in December 2014, the first since 2001. The Region's Polio LabNet continues to have very high performance with all laboratories passing the virus isolation proficiency test (PT) and the ITD PT. Two countries experienced challenges with the 2014 sequencing PT and are currently addressing the issues identified.
A phased implementation of poliovirus containment following GAP III is planned and all countries are requested to update their inventories with type specific information. Biorisk management training will be held for laboratory directors, national containment coordinators, national regulatory containment coordinators and IPV manufacturers and research and development facilities.

2.1.4 Methodologies for VDPV detection, characterization and virologic classification

The most recent cVDPVs reported are: type 1, September 2014 in Madagascar; type 2, 2014 in Pakistan, Nigeria and South Sudan; type 3, July 2013 in Yemen.

There has been limited circulation of cVDPV2 in Nigeria and Pakistan in 2015 and these were found only in the environmental samples. In Kaduna, Nigeria, there is clear evidence of circulation from the ES but no AFP cases have been detected.

Assays for detecting VDPVs are in development by the United States Centers for Disease Prevention and Control (US CDC) and the new “rule-in” assay directly targets VDPVs and will reduce the high false positivity rate of the old “rule-out” assay. The rule-in VDPV assay is very good at detecting VDPVs directly from stools. The rule-in assay could replace the rule-out assay or possibly be used in conjunction.

VDPV investigation in the Philippines in 2015

The Philippines last detected cVDPV3s (N=3) in July 2001 in AFP cases in Luzon and Mindanao. No further cases were found after a national immunization activity was held. A new VDPV was detected in December 2014 in Mindanao from a 4-year-old female with AFP. The child had received OPV 3 doses in 2010 and a measles rubella (MR)-OPV campaign was held in September 2014 in that part of the country. In response to the case, samples were collected from healthy children contacts. More samples were also collected from the case. No further VDPVs were found. Next steps are: the need for comprehensive improvements in sensitivity of surveillance and response, improving the cold chain, and updating WPV/VDPV preparedness plan.

VDPV surveillance in China

China has established high quality AFP surveillance with a strong network of polio laboratories. It has detected two cVDPV outbreaks (Guizhou and Sichuan), five iVDPV cases and more than 30 aVDPV cases from 1997 to 2012. These outbreaks were associated with only one to three AFP cases viruses which were all newly emergent VDPVs, 0.6% to 2.2% nucleotide differences in the viral capsid protein 1 (VP1) region, and the phylogenetic analysis implied that the cVDPVs circulated for less than one year following the initiating OPV dose. In 2014, two aVDPV2 cases and one iVDPV3 case were detected. Sequential samples collected from the iVDPV case detected no further virus. In 2015, one case of aVDPV1 from a healthy child was detected and two iVDPV2 cases and one aVDPV1 from AFP were detected. Evidence shows that surveillance is effective at detecting VDPVs and transmission is limited through a strong immunization programme. China plans to withdraw OPV2 in sync with the rest of the world and would prefer to use IPV for the entire 15 million birth cohort but does not have sufficient stocks for the whole country.

2.1.5 Country reports

Australia

Australia has developed a WPV response plan which was revised in 2014. The country switched from OPV to IPV in 2005 and a WPV1 imported from Pakistan was found in 2007. Sabin-like polioviruses were detected from two AFP cases (2009 and 2013) and two environmental samples (2015). Stool collection rates have always been a challenge and difficult to reach the target. AFP surveillance has been enhanced by doing enterovirus surveillance and ES. The Victorian Infectious Diseases Reference Laboratory (VIDRL) supports Brunei Darussalam (since 2002), Papua New Guinea (since 2002) and Pacific island countries (since 1993) for AFP testing. No polioviruses have been detected and an
average non-polio enterovirus (NPEV) rate of 20% for the Pacific island countries and 38% for Papua New Guinea was reported.

**China**

More than 10,000 stools from 5000 AFP cases are tested by the China LabNet annually with an adequacy rate of more than 90% every year since 2000. Though the number of polioviruses isolated per year has gradually declined since 2002, the NPEV rate is constant at around 10% every year. The new polio ITD was introduced in January 2013 with 23 provincial laboratories performing RT-PCR. All passed the ITD and VDPV PT for 2014. The other eight provincial laboratories (except Tibet) have now finished the two quality assurance steps and will complete ITD in their own laboratories shortly. All 31 provincial and national polio laboratories passed the global isolation PT in 2014 and the national laboratory passed the sequencing PT. Capacity of the LabNet is maintained through regular training. Training in September 2014 focused on use of the new algorithm to improve reporting timeliness to 14 days in 2015. Accreditation review of six to eight laboratories is carried out each year by a team of international experts, and all provincial and national laboratories are currently fully accredited. Nine provinces carry out ES and two more will start shortly.

**Hong Kong SAR (China)**

Hong Kong SAR (China) moved to IPV in the form of Diphtheria, Tetanus, Pertussis and Polio (DTaP)-IPV in 2007 and since then poliovirus detection in AFP and other surveillance samples has been very low. Polio serosurveillance is carried out every five years and in 2010, immunity to all three serotypes was in excess of 90% except for the 21 to 30 year age group for type 3. IPV-only immunized children had similar immunity levels to OPV immunized children one year after IPV was introduced. A national action plan for detection of and response to WPV importation and cVDPV has been developed. The polio laboratory, housed in the Public Health Laboratory Service, has met all the GPLN performance and quality indicators and is functioning at biosafety level 2; however the Institution has biosafety level 3 capability.

**Japan**

Japan changed from OPV to IPV in 2012 and is using the locally produced DTaP-sIPV (Sabin IPV) and also DTaP-cIPV (conventional IPV) from Birken. A national action plan for detection of and response to WPV importation and cVDPV has been developed.

NIID is a global specialized laboratory and uses seroprevalence, enterovirus surveillance and ES in addition to AFP surveillance in Japan. The immunization status of under 5 olds with different immunization schedules, OPV, IPV (c and s) or OPV and IPV were examined while those immunized with IPV only appear to have equivalent antibody response to those immunized with OPV only or a combination of both. NIID supports AFP surveillance in Cambodia and the Lao People's Democratic Republic and provides reference activities for Mongolia, the Republic of Korea and Viet Nam national laboratories. The NPEV rate for Cambodia and the Lao People's Democratic Republic was more than 20% in previous years but has declined recently.

Data analysis of the sequencing PT was not appropriate and needs to be standardized. NIID suffered a penalty because of this. NIID facilitates annual Japan International Cooperation Agency (JICA) training for polio and measles and has initiated technical assistance to national polio laboratories in Nigeria.

**Malaysia**

Malaysia has a population of 31 million with approximately 9.5 million under 15 years and expected 95–96 AFP cases per year. The non-AFP rate is 1.5–2 and approximately 160 AFP cases are detected per year. A national action plan for detection of and response to WPV importation and cVDPV has been developed. IPV is used for the first four doses (2, 3, 5, 18 months) and an OPV booster is given at 7 years.
The polio laboratory is situated in the Institute for Medical Research (IMR) and fully accredited since 1998. The laboratory has been accredited for ITD since May 2010 but has not yet been approved for sequencing. The laboratory has a low NPEV but use HFMD surveillance and have high enterovirus detection rate. From 2012 to 2013, there is no poliovirus isolated from AFP surveillance, but polioviruses were detected from ES.

**Mongolia**

Mongolia has a population of 3 million with 852 000 under 15 years. A national response plan for WPV importation was developed in 2014. Mongolia has applied to Gavi, the Vaccine Alliance for IPV introduction. Routine OPV coverage was reported as 96% in 2014. Approximately eight cases of AFP are detected per year and the NPEV rate was 12.5% in 2014. Healthy children surveys have been carried out each year from 2013 to 2015. No poliovirus has been identified since 2012. The laboratory’s performance indicators meet all requirements and the laboratory is fully accredited. ES is planned for introduction in Ulaanbaatar (population 1 million) and will be implemented after appropriate training.

**New Zealand**

New Zealand’s population is 4.4 million with 800 000 under 15 years and eight to nine AFP cases are expected per year. A national response plan for WPV importation was developed in 2009. OPV was replaced by IPV in 2002 and Sabin virus was shown to disappear very quickly over six months through ES and enterovirus surveillance. Three polioviruses have been detected since then two of which were associated with OPV vaccine directly or through a contact. One had unknown origin. AFP case detection was low until 2012 when AFP surveillance was introduced into the largest children’s hospital in the country and AFP detection was improved. NPEV rates exceed 10% for the past four years and all laboratory performance indicators meet the minimum requirements. Sequencing was established in 2014 and the laboratory gained 100% in the sequencing PT.

**The Philippines**

The Research Institute for Tropical Medicine (RITM) was established as the national polio laboratory in 1998 and has since remained fully accredited. It was accredited as ITD and VDPV detection laboratory for RT-PCT in 2014. Non-polio AFP rates have been declining since 2007 and have been approximately 0.7/100 000 for the past two years. The laboratory's NPEV rate for 2014 was 3.3% after reaching almost 10% in 2013. A rate of 3.6% has been detected for 2015. The Philippines developed a preparedness plan. The laboratory actively participated in the plan's development. The plan covers enhancement of surveillance, laboratory, and immunization in the event of a laboratory-confirmed WPV being detected. IPV as a single dose was introduced in the third quarter of 2014 and in a phased manner to the rest of the country in 2015. RITM is carrying out HFMD surveillance but it has not yet been introduced HFMD into the disease surveillance programme. To improve NPEV rates, a study is underway to determine NPEV in healthy children under 5 years. Early results look promising and more than 10% of children have NPEVs detected. Two polioviruses were also detected from one site in the national capital region. ES is being considered for the country and RITM staff have been trained. The logistics and financial support required are being determined.

**The Republic of Korea**

The Republic of Korea has a population of 51 million and more than 7 million are under 15 years. IPV was introduced in 2005 and coverage is more than 95%. AFP surveillance is through 50 Paediatric Neurology AFP surveillance hospitals throughout the country. The non-polio AFP rate has exceeded 1/100 000 since 2012 and enterovirus EV71 is the most common cause of AFP. The national laboratory at Korea Centers for Disease Control and Prevention (KCDC) meets all the performance indicators and is fully accredited and was additionally approved for performing ITD in 2014 using RT-PCR. A revised national action plan for polio importation was developed in 2012.
Singapore

Singapore's AFP rate has been over 1/100 000 for the past two years. Polio vaccine is administered as IPV for four IPV doses (3, 4, 5 and 18 months) and OPV at 10–11 years. Reported coverage is more than 90%. The National Polio Laboratory is the diagnostic laboratory for Singapore General Hospital and has been accredited since 1998. In addition to AFP, surveillance is also carried out through non-AFP, non-stool and ES of raw or treated reservoir or river water, not sewage. The laboratory is operating at a high level of performance and is accredited for performing ITD.

Viet Nam (Hanoi)

Polio campaigns have been held in 2011, 2012 and 2014 with coverage of more than 97%. Routine coverage is reported as more than 93%. The AFP rate has exceeded 1/100 000 since 2011 and more than 96% have adequate stools. The laboratory has achieved an NPEV rate of more than 10% since a low of 5.4% in 2010. The laboratory established the new algorithm in 2011, passed the isolation and ITD PTs and is fully accredited. New human rhabdomyosarcoma (RD) a mouse cell line (L-cells), genetically engineered to express the human poliovirus receptor (L20B) were received in 2012 and a mycoplasma test received from NIID was negative.

Viet Nam (Ho Chi Minh City)

The Ho Chi Minh City national laboratory is responsible for 20 provinces in the southern half of the country. AFP surveillance from 2014 to 2015 identified more than 10% NPEVs and no polioviruses were detected. The laboratory staff were trained in ITD and achieved a passing score in the 2014 PT. ITD was done on two L20B isolates in 2013 and none in 2014. Samples will be practiced monthly for ITD to maintain expertise.

2.1.6 Polio recommendations from the Fourth Meeting on VPD LabNet in the Western Pacific Region

The regional VPD LabNet meeting in March 2013 made 27 polio-specific recommendations. Almost all (70%) were completed by the LabNet. Of the partially implemented recommendations (30%) most were 80–90% completed. Some laboratories had challenges in meeting reporting timeliness. A small number of countries reported difficulty in shipping samples to the reference laboratory for confirmation within the correct timeframe. The access database for data management was used by most laboratories. Reporting non-AFP data was incomplete for some countries with extensive enterovirus surveillance systems with large numbers of results. A summary of the accreditation status of the polio LabNet (Table 1) showed the very high level of performance by the laboratories. All met the minimum performance indicators and all were fully accredited.
Table 1. Summary of accreditation and performance of polio national laboratories

<table>
<thead>
<tr>
<th>Laboratory</th>
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<th>ITD PT 2014</th>
<th>Sequencing PT 2014</th>
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2.1.7 Quality assurance in the Western Pacific Region

A review of the most recent virus isolation PT test was reported. Each annual PT panel consists of 10 stool samples with single or combination of polio and enteroviruses. Some samples may be negative. Reporting is required within the standard 14 days and laboratories must attain 90% to pass. Any laboratory which fails to pass requires a full investigation of their worksheets to identify deficiencies to resolve any problem. A new PT is then provided. In early 2014, all 43 polio laboratories in the Region received the 2013 panel. All laboratories in the Region passed with 37 scoring 100%, four scoring 95% and two scoring 90%. PT sample 10, which contained coxsackievirus (CVA)16, was found to be the most challenging. One laboratory in another WHO region was found to have contaminated their L20B cells with RD which gave spurious results.

2.1.8 Proficiency testing for polio molecular diagnostic methods

The PT for polio molecular diagnostic methods tests the proficiency of GPLN ITD laboratories in RT-PCR, including interpretation and reporting, use to field-test the reliability and durability of polio molecular reagents and helps to identify GPLN training needs. The scoring is based on the accuracy of the final result with deductions for technical issues and timeliness of reporting. A planned new scoring system will more heavily penalize failure to detect WPV and VDPV. In 2014, 31 laboratories in the Region received the ITD PT and under the old scoring all laboratories passed. However if the laboratories had been assessed under the new scoring scheme only 21 of 31 laboratories would have passed. For the 2014 sequencing PT, seven laboratories in the Region participated and two failed with scores of less than 90% (83% and 84%). For the failed laboratories, extensive troubleshooting and training has occurred and a repeat PT will be provided.

2.1.9 Cell authentication and introduction of FTA cards for shipment of cell lysate

Inadvertent cross-contamination of cell lines is a major problem in some laboratories and was identified in one laboratory participating in the global virus isolation PT. Cross contamination can arise from poor handling techniques or sub-culturing different cell lines at the same time. It may not be possible to identify contamination morphologically. Cross-contamination of cell lines can lead to misidentification of virus isolates. Several methods can be used to confirm cell line origins, including melt curve analysis and RT-PCR for human and mouse genome to identify mixtures of L20B and RD. Flinders technology associates (FTA) cards can be used to safely transport nucleic acid samples to reference laboratories. Samples should be heated at 70°C for five minutes to inactivate any infectious
material and can be shipped via FedEx or other courier. The VIDRL cell repository was confirmed to be non-reactive to foreign cell lines.

2.1.10 Update on the new ITD algorithm and CDC rRT-PCR assays ver. 4.0 for ITD and VDPV screening

The sensitivity of the ITD version 3.0 method needs to be increased for environmental and/or direct stool testing. Various commercial kits were compared to the current CDC ITD kit and some were found to be more sensitive and also cheaper. The commercial ToughMix kit increases sensitivity of detection, especially with mixtures and signals are easier to interpret. The ITD v4.0 with ToughMix has fewer discordant results due to not using the serotype assays (which sometimes give weak/negative results with virus mixtures), instead relying on the Sabin and WPV specific assays. Because of the increased sensitivity any issues with laboratory techniques will become obvious. The v4.0 ITD kit has been shipped to the WHO Regional Office for the Western Pacific and is ready for shipment in the second quarter of 2015. Some issues will likely arise as it is used in the field. These will be addressed through the continuous update of primers.

A new ITD algorithm was developed to improve sensitivity and specificity to quickly identify viruses for sequencing and for direct screening during outbreaks which has fewer discordant results due to not using serotype assays which may fail in mixtures. The new algorithm is more sensitive to detect more Sabin viruses and will be useful for detecting Sabin 2 in environmental samples.

2.1.11 Environmental surveillance and plans for expansion plan and guidelines

Environmental surveillance (ES) which is used to supplement AFP surveillance increases the sensitivity of the surveillance system, especially in areas where AFP surveillance is under-performing. Following OPV2 withdrawal, there is a need to detect Sabin 2 viruses which may still be circulating and AFP surveillance alone is not sufficient to detect Sabin viruses. ES provides supportive data for the certification of polio eradication. Polio Eradication and Endgame Strategic Plan 2013–2018 proposes environmental sampling sites in at least 20 additional cities and locations globally, prior to the switch to bOPV in 2016.

New countries planning to introduce ES include Democratic Republic of Congo, Kenya, Madagascar, Nigeria and Somalia. Iraq, Syria and Yemen will introduce ES by the end of 2015.

Current recommended sampling is using the grab process and double phase separation for purification. Wastewater treatment plants are good sites for ES but in their absence sampling drainage from large population areas is helpful. One promising sampling method uses a filter/bag method developed by University of Washington. A 10 litre filter bag directly concentrates material onto the filter which can then be shipped to the laboratory for elution.

The new ES guidelines are available on the Expanded Programme on Immunization (EPI) website and participants are requested to provide feedback.

Environmental surveillance in Australia

Australia has established ES in Melbourne and in regional New South Wales to enhance polio surveillance due to perceived gaps in AFP surveillance. Collection sites are situated at waste water treatment plants and 800 ml raw sewage grab samples are taken and concentrated using standard WHO methods. No polioviruses were detected from 36 samples collected in New South Wales from 2010 to 2012 and PV2SL and PV3SL were detected from 21 samples collected from 2014 to 2015.

Environmental surveillance in China

Nine provinces in China have established ES including Xinjiang, one of the three provinces considered at high risk of importation of WPV. Tibet is also considered a high risk province but it has been challenging to establish ES there. China uses the grab method for collection and the Katayama
negatively charged membrane/filtration concentration method. Results from the nine sites from 2011 to 2015 identified 2135 poliovirus, all of which were Sabins except for VDPV2 detected from single samples from Shandong in 2012, Guangdong and Heilongjiang in 2013, and Fujian, Guangdong, Heilongjiang and Xinjiang in 2014.

**Environmental surveillance of poliovirus and non-polio enteroviruses in Malaysia**

ES was established in Malaysia in January 2012, in states with less than optimal AFP rates and immunization coverage of less than 95%. Three states were selected, each serving 100–300 000 population, Sabah, Selangor, and Kuala Lumpur (although Sabah is no longer used due to logistics issues). Staff are trained at NIID and the National Institute for Biological Standards and Control (NIBSC) and use the standard WHO protocol: two phase separation and passage into RD and all positives are tested by new algorithm and ITD and sequencing. Polio Sabin-like viruses were found in all sites between 2012 and 2014, with all serotypes detected and also NPEVs.

**Environmental surveillance in Japan**

Japan started ES in 2013 and used it around the period of introduction of IPV. No Sabin virus was detected from ES after the introduction of IPV in 2012 to 2013. A total of 13 sites have been selected; eight national and five research institutes. The method used is negatively charged membrane and any virus detected is shipped to NIID for ITD and further analysis. Results show that multiple enteroviruses were detected, but no PVs. NIID investigated the use of magnetic nano-beads to concentrate virus from sewage. Beads are coated with human anti-PV antibody and mixed with treated sewage, and washed to concentrate. Sensitivity is acceptable but cost of beads is relatively expensive, however NIID can provide small scale material to network laboratories to evaluate.

**HFMD surveillance in China**

An enterovirus 71 (EV71) outbreak with high mortality rate emerged in 2008 in Fuyang, Anhui Province, China with an initially high mortality rate (12/12). The outbreak ultimately resulted in 6049 cases with a mortality of 2.75%. Since 2008, a nationwide outbreak has occurred every year in China. Currently more than one million cases HFMD a year with 905 cases dying in 2010. Cases peak in spring. All prefecture laboratories are capable to perform PCR detection. Of the different viruses causing HFMD, 70–90% of fatal cases are infected with EV71 with the highest mortality in ages 6 months to 3 years. A vaccine has been developed and approved for use in China.

**NPV and HFMD surveillance in Japan**

EV71 and coxsackievirus A16 (CVA16) give classic HFMD clinical manifestations and CVA6 can give atypical HFMD, higher fever, and expanded blisters. EV71 can induce a polio-like paralysis. Cambodia, China and Viet Nam have experienced large outbreaks of HFMD since 2008 with high mortality. In Japan, large outbreaks of HFMD occurred in 2011 and 2013, with EV71, CVA16, and CVA6 the predominant infectious agents detected. Japan has developed an EV71 vaccine which is in phase III clinical trials. A human parechovirus (HPeV) type 3 outbreak was detected in Japan in 2014 with fever, diarrhoea and meningitis symptoms predominant.

**HFMD and laboratory surveillance in northern Viet Nam**

The National Institute of Hygiene and Epidemiology (NIHE), Hanoi, serves 28 provinces for HFMD surveillance. In 2008, a small HFMD outbreak in northern Viet Nam with circulation of EV71 subgenotype C5 (22.5%) and CVA16 (74%). HFMD surveillance in Viet Nam detected: 113 131 cases in 2011; 157 391 cases in 2012; 78 818 cases in 2013; 80 868 cases in 2014. However most cases and deaths were in the south. The most affected age group was children under 3 years. The disease occurred in all provinces of northern Viet Nam (delta and mountainous areas) with co-circulation of EV71, CAV-6 and CAV-16 in 21/28 provinces, EV-71 and CAV16 in five provinces, EV71 and CAV6 in one province.
2.1.12 Introduction to GPLN management system (GPLNMS) and plans for implementation

The GPLN management system (GPLNMS) mission is to improve coordination by addressing gaps in the global management of information. The aims are to: provide comprehensive capture and archiving of laboratory data generated by the GPLN; streamline key processes (annual reporting, accreditation, PTs); develop online monitoring tools (facilities, equipment, staff, exchange of materials); provide a space and forum for standard documents, discussion and information exchange. Electronic sharing of information with multiple partners is necessary and the GPLNMS will gather all laboratory information in one place. There will be a requirement to submit information on staff, equipment and supplies, an annual report and the accreditation report. Annual and accreditation indicators will be computed from laboratory files received at WHO headquarters in order to validate values/information reported by the laboratory in GPLN and PTs (VI, ITD and sequencing) will be added.

2.1.13 Polio laboratory data management

Current status

Data is collected weekly from the Regional Polio LabNet. The polio data exchange file is sent to WHO headquarters. A bi-weekly polio bulletin is distributed via email and uploaded on the WHO Regional Office for the Western Pacific website; and the quarterly polio laboratory summary is sent to WHO headquarters. The Access Polio Laboratory database was released during the fourth VPD LabNet meeting and was intended to guarantee all core variables are being reported, to improve data quality and to standardize the reporting format. Currently 10 of 12 laboratories are using the database. These laboratories cover 13 of 16 countries. Japan and Singapore are not using the database but are reporting using the old database. A new Polio AFP Surveillance and Reporting System (PASRS) is a web-based surveillance and reporting system which is in development and should be ready by the end of 2015. All levels of surveillance will access the database (surveillance, NL, RRL, GSL). Data can be entered at any time and is immediately reported to the Regional Office (no need for a feed forward process). Once the AFP data is entered by the AFP surveillance programme an email is sent to the laboratory notifying them to enter data.

Roundtable sessions: group discussion on laboratory management issues

Participants were divided into three discussion groups:

1) Contingency planning in Western Pacific Region's polio laboratories: The purpose of contingency planning is to help ensure rapid, appropriate and effective crisis response, with a focus on exploring general scenarios and the systems that are in place to respond to them. The key steps in the process include: analysing the potential hazard and risks; identifying, defining and prioritizing contingencies; developing scenarios for the planning process; preparing a plan for each selected scenario; and maintaining and updating the plan.

2) Data management and timeliness of reporting: The WHO Regional Office for the Western Pacific Access database was evaluated by the regional LabNet members. It was determined to be beneficial for laboratories which use the Access database, however, there was a need for auto-generated reports; country-specific reports; and an interface with the laboratory database. The reporting of non-AFP and environmental data is required on a monthly basis in an aggregated format. Some laboratories will experience extra workload in submitting data from large surveillance programmes but collaboration with the data managers in the country and the Region may be able to facilitate reporting.

3) Quality assurance and quality control: To gain the greatest benefit from performing quality assurance (QA) in the LabNet, guidelines could be developed for trend analysis for cell sensitivity testing in situations where controls are persistently under or over the mean, though still within the limits. To ensure that cell quality is high and sensitivity maximized, mycoplasma testing should be regularly carried out (late in their passage) and used to confirm that frozen cell stocks are clear of infection. Cell authentication should be carried out on cell stocks held by laboratories acting as cell
repositories. The authentication may be carried out by National Institute for Biological Standards and Control (NIBSC) or other facilities approved by the Global Laboratory Coordinator and FTA cards can be used to facilitate shipment. For low workload laboratories there is a need to maintain proficiency by testing stored isolates. For countries experiencing shipping problems, laboratories could use FTA cards and work with the country office to facilitate clearance. Containment of poliovirus will enter a new era once OPV2 is withdrawn. Laboratories will be required to complete inventories prior to doing containment and need to plan for removing all type 2 reference strains from all testing.

2.1.14 Global and regional update on eliminating measles and rubella

The Western Pacific Region has established measles and rubella elimination goals with a measles target of 2012. For rubella a target date has yet to be set. Measles containing vaccine (MCV1) coverage for the Western Pacific Region was reported as 97% in 2013 and MCV2 of 92%. Globally MCV1 was reported as 84% and MCV2 as 53%. Globally great progress has been made with the number of measles cases showing a reduction of 72% from 2000 to 2013 with incidence reducing from 140 to 40 per million. However the incidence rate is still eight times higher than the global milestone to be achieved by 2015, which should be less than five reported measles cases per million. Regional measles incidence (per one million) has continued to rise from 5.9 in 2012 to 17.7 in 2013 and 44.0 in 2014. This can be attributed to resurgence of endemic transmission, re-established endemic transmission, large-scale outbreaks induced by importation, and multiple importations resulting in increased measles incidence.

Seven countries and areas in the Region, (Australia, Brunei Darussalam, Cambodia, Japan, Macao SAR (China), Mongolia, and the Republic of Korea) have been verified to have eliminated endemic measles. Hong Kong SAR (China), New Zealand, and Singapore are considered close to verification.

Outbreaks in China (endemic), Mongolia (imported), and the Philippines show a high proportion of cases in the very young and in young adults. Current strategies for measles elimination have not addressed measles infection and transmission among very young children and adolescents and young adults.

For rubella, only three countries have yet to introduce rubella containing vaccine (RCV) into the national immunization programme as of 2014: Papua New Guinea, Vanuatu and Viet Nam. Reported cases have declined from 76 000 to 13 000 since 2011. China, Japan and Viet Nam report the largest numbers of cases in the Region and both Japan and Viet Nam have established CRS surveillance. In 2011, reported CRS cases totalled 200: Cambodia (N=9), Singapore (N=2) and Viet Nam (N=189). In 2014, a total of 12 cases were reported: Cambodia (N=3) and Japan (N=9). However as CRS surveillance is not functional in countries with large populations and is only sentinel in other countries, it is estimated that the regional burden is approximately 9000 per year (2010). Australia, New Zealand, the Republic of Korea and Singapore are approaching rubella elimination. Japan’s target is to eliminate rubella by 2020. Mongolia aims to eliminate rubella by 2020.

2.2 Global measles and rubella LabNet

The global measles and rubella LabNet continues to function at a high level of accuracy and high throughput. A total of 96 103 measles samples (71% from Western Pacific Region laboratories) were confirmed from 367 540 (57% from Western Pacific Region countries) suspected measles cases in 2014. Additionally, in 2014 the LabNet tested 106 987 specimens for rubella, 12 025 of which were positive. The quality of the LabNet remains high with no laboratory failing the global PT in 2014 and most are fully accredited.

There are some laboratories with measles not obtaining or not sharing their measles and rubella sequence data with the LabNet through the sequence databases, Measles Nucleotide Surveillance (MeaNS) and Rubella Nucleotide Surveillance (RubeNS). In 2014 only 47% of countries reporting
measles cases submitted genotype information. For rubella only 7% of countries with laboratory-confirmed rubella reported rubella genotype information. India is now sharing data regularly.

Recent developments have seen the introduction of a molecular PT programme and workshops for the molecular detection and genetic characterization of measles and rubella viruses. Revision of the laboratory manual, a seroprevalence assessment guideline, standardization of rubella IgG testing, vaccine specific measles PCR and using an extended sequencing window for measles virus are all underway. Constraints identified include: data timeliness and completeness, CRS surveillance, private laboratories completeness of surveillance data, increasing workload for LabNet, elimination goals now in all WHO regions, requirement to perform additional laboratory tests in countries with low incidence of measles and/or rubella.

The Western Pacific Regional Measles and Rubella LabNet consist of 386 laboratories covering 37 countries and areas. The LabNet has 48 of 52 laboratories fully accredited with one pending and three Pacific island countries laboratories requiring a visit. All 53 laboratories passed the global serology PT in 2013 and 2014 and the four reference laboratories all passed the global molecular PT.

The workload increased dramatically in 2014 for the non-China laboratories, increasing from an average of approximately 20 000 a year from 2010 to 2013 to more than 50 000 in 2014. Most of the increase in testing was due to big measles outbreaks in Papua New Guinea, the Philippines and Viet Nam. The China LabNet workload also increased to more than 100 000 tested for measles in 2014. Timeliness indicators were affected by large outbreaks in the Philippines and Viet Nam, the subsequent shortage of test kits and the reluctance of some countries to use EPI-linking overloaded the laboratories.

Molecular surveillance data was reported by 12 countries in 2014 and five in 2015 (April). H1 continues to be the predominant genotype in China but was also found in Australia, Hong Kong SAR (China), Japan, Macao SAR (China), Malaysia, the Republic of Korea, Singapore and Viet Nam. B3 was predominant in 2014, the cause of outbreaks in Japan, New Zealand, Papua New Guinea, the Philippines and the Republic of Korea and also detected in Australia, Hong Kong SAR (China), Malaysia and Singapore. D4 genotype has almost disappeared in the Region over the past four years. D8 has made a resurgence after almost disappearing and was found in Australia, Japan, Mongolia and the Republic of Korea's outbreak which was caused by H1, imported from China. In 2014 rubella genotypes were reported from Malaysia (1E, 2B), New Zealand (2B) and China (1E, 2B).

In summary, the Western Pacific Region has a highly proficient LabNet with strong quality assurance and provides accurate and timely laboratory confirmation and genotyping evidence to the programme. Both the proportion of laboratory-confirmed and genotyped cases increased in 2013 to 2014. The Hong Kong SAR (China) regional reference laboratory hosted two hands-on trainings. These trainings strengthened molecular capacity and cell culture and virus isolation capabilities. For the countries that have verified measles elimination, genotype evidence supports the interruption of endemic measles virus transmission.

2.2.1 Global specialized laboratories (GSLs) and regional reference laboratories (RRLs)

Japan as GSL

The global specialized laboratory at NIID, Japan continues to be involved in strengthening the capacity of the LabNet. The laboratory helped facilitate the JICA global training in measles and rubella in 2015 and carried out seroepidemiology for measles and rubella in the Lao People's Democratic Republic using dried blood spot (DBS).

Commercial laboratories perform the bulk of IgM testing for measles and rubella in Japan and prefectural laboratories perform PCR and sequencing tests.
In 2014, 2210 suspected measles cases and 984 suspected rubella cases were tested by PCR and positives were 417 and 29 respectively. To build capacity and ensure quality in national surveillance LabNet NIID has revised the manual for measles and rubella, developed a molecular PT with 22 laboratories and developed liaisons with five of the largest commercial laboratories where they now share data and report performance. Lack of samples limits NIID in establishing a PT for the commercial laboratories but the laboratories are ISO accredited and undergo College of American Pathologists QA programme.

A total of 463 measles cases were reported in 2014 and 20 in 2015 (May). Genotype B3 was predominant from 2013 to 2015 with 72% identical to one strain, “Fukuoka 20.13”. Three other genotypes were detected from 2012 to 2014; D8, D9, and H1. D9 makes up 25% of 12 strains detected in 2015, compared with 6% of strains in 2014.

Australia as RRL

Australia has undergone verification of measles elimination. Molecular evidence was key to the decision-making and showed that measles is detected all year round, multiple diverse range of genotypes are identified (B3, D4, D5, D8, D9, G3, H1). Circulation of a single genotype has not been sustained for longer than 33 weeks (D8 outbreak in 2012); chains of transmission are small (median number of cases is less than 4, upper range less than or equal to 25) and of short duration (60% of all chains lasted less than or equal to 21 days) and 480 of 567 (85%) of confirmed cases were imported or linked to imported cases. All evidence satisfies the criteria, which is consistent with a country that has eliminated endemic measles.

Australia reported 340 measles cases in 2014 and 37 in 2015. Rubella positive cases were 17 for 2014 and five for 2015 (8 May). Two CRS cases were notified in 2013 and none in 2014 and 2015. The distribution of measles genotype detected in 2014 showed that virus was detected in 47 of 52 weeks, six genotypes were identified, with B3 most common. However the B3 was proven through epidemiological investigation to be due to multiple importations from the same source rather than continuous circulation.

VIDRL supports the Region by testing suspected case samples from Papua New Guinea, Samoa and Solomon Islands and also supports the confirmatory testing of the New Zealand national laboratory. VIDRL has developed a measles RT-PCR to detect vaccine strains (genotype A), which will be helpful in the global LabNet.

China Measles LabNet (with 31 provincial laboratories)

A total of 52 450 measles cases were reported in China in 2014, almost twice as many as the 27 769 cases reported in 2013. As of May 2015 a similar pattern to 2014 is emerging. For rubella, 2691 cases tested positive for rubella IgM from 102 574 suspected sporadic cases. For measles virus surveillance, a total of 11 283 viruses were detected from 1993 to 2015. Nine genotypes were detected but in 2014, 99% were H1 with B3, D8, D9, G3 found additionally. In 2015 viruses were detected from 21 provinces. The quality of the laboratories in China is high. In 2014, all 32 provincial and national laboratories passed the global PT. Six to twelve provincial laboratories undergo on-site reviews by international experts annually since 2000 and the pass rate is very high.

Hong Kong SAR (China)

Surveillance of local cases found 50 measles and 15 rubella-notified cases in 2014 compared with 38 measles and 25 rubella cases in 2013. There has been an increasing number of measles cases while there is a reduction in the number of rubella and mumps. Prior to 2009 no H1 genotype was detected however it is now the predominant genotype detected in 2014, 29/42 (69%). Genotypes B3 (21%), D8 (7%) and D9 (2%) were also detected in 2014. Rubella genotypes included 2B (N=2) in 2014 and 1E (N=1) in 2015. The laboratory reported to have switched to using molecular tests for measles virus detection but still maintain their expertise in virus culture.
The laboratory has a critical role for monitoring the performance of nine national laboratories in the Region: Cambodia, the Lao People's Democratic Republic, Macao SAR (China), Mongolia, Papua New Guinea, the Philippines, Singapore and Viet Nam (N=2). All have shown good concordance rate with the reference laboratory. In 2014 and 2015, 147 measles sequences were determined from serum samples from seven countries. A total of 16 sera from the 2015 Mongolia outbreak were all positive for H1 genotype. Finding rubella sequences from serum samples was less successful than for measles, however 21 sequences from four countries were found from 2014 to 2015, Cambodia (2B), the Philippines (1J and 2B), Singapore (2B), and Viet Nam, Hanoi (2B).

2.2.2 Global distribution of measles genotypes

Updating the measles nomenclature is planned for later in 2015 and will be published in the Weekly Epidemiological Report. The sequences of the measles virus Edmonston strain and other vaccine strains will be updated and submitted to MeaNS. Analysis of MeaNS shows that four key genotypes in 2014; B3, D8, D9 and H1 with D4, D6, and G3 also reported. Most submissions to MeaNS are from the Western Pacific Region and China in particular. The concept of using a “named strain” for strains detected is not being fully utilized. This makes it challenging to identify when the same strains are circulating widely. Participants were encouraged to nominate strains when they exceed 50 strains with identical N450 sequences. For example, the named strain “B3 Harare.ZWE/38.09” have spread globally and have been found in 10 countries since 2009, eight of them in 2014.

2.2.3 Regional molecular surveillance update

In the Western Pacific Region, genotype H1 predominates due to the large number of cases in China and the excellent molecular surveillance carried out there but also through its spread to neighbouring countries in the past five to six years. However, prevalence of B3 has been increasing up to 2014 and been found in outbreaks in Australia, Japan, New Zealand, Papua New Guinea, the Philippines, the Republic of Korea, Singapore and Viet Nam. In 2015 (April) D8 appeared to be predominating (five countries) and fewer B3 strains (two countries) were reported. For the countries which have verified measles elimination, genotype evidence supports the interruption of endemic measles virus transmission. In endemic countries: replacement of genotypes or mixed genotype pattern is apparent. The proportion of genotyped cases has increased from 2013 to 2014 but could be improved.

2.2.4 Harmonizing sequence data

There is a discordance of data used for generating genotype maps between WHO headquarters and the WHO Regional Office for the Western Pacific and MeaNS and RubeNS. For example in 2014, 5401 sequences were reported to MeaNS by Western Pacific Region countries but 5914 strains (9.5% more) were reported in the WHO Western Pacific Region Measles Bulletin. Some countries had a small percentage difference and some a large discordance (Japan, New Zealand, Singapore and Viet Nam). There is a need for timely reporting and harmonized databases. Preferably only one database (MeaNS) should be used.

2.2.5 Update of the Fourth Meeting of the Regional Verification Commission

There are three criteria for verification: documentation of the interruption of endemic measles virus transmission for a period of at least 36 months from the last known endemic case; in the presence of verification-standard surveillance; and genotyping evidence that supports the interruption of endemic transmission. Seven countries and areas were verified in 2014/2015 as having achieved elimination: Australia, Brunei Darussalam, Cambodia, Japan, Macao SAR (China), Mongolia and the Republic of Korea. A further 10 countries have provided reports with detailed information on progress towards measles elimination – five may be ready for verification but require further information and five have ongoing transmission. Verification progress or achievement is reviewed on an annual basis. It is considered that total measles incidence is a useful measure of progress towards elimination but is not required.
2.2.6 The role of the LabNet to support verification of measles elimination

In support of verification of measles elimination, LabNet provides seroprevalence assessments, and analysis of genotype information and the completeness of viral surveillance. The Regional Verification Commission expressed concern about the inconsistency and variability in seroprevalence data and the apparent lack of a standard method for conducting seroprevalence studies. However, the LabNet has a working group that is preparing guidelines for seroprevalence studies and draft guidelines should be circulated soon. For the analysis of genotype information a phylogenetic analysis of the sequences from all cases that have been genotyped is expected and these should be matched to named lineages in MeaNS to help identify transmission chains. Verification requires proof of absence of any endemic transmission.

2.2.7 Country presentations

All countries presented a summary of their achievements. All laboratories passed the global PT, have acceptable concordance with their confirmatory tests and are fully accredited with the exception of Viet Nam, Ho Chi Minh City, which is pending and waiting for their current confirmatory test results, as per Table 1. Most countries presented their testing and reporting algorithms which followed WHO recommendations. Two countries (the Philippines and Viet Nam) experienced delays in meeting reporting timeliness due to big outbreaks experienced in their countries overwhelming testing capacity. Both also experienced kit shortages which impacted reporting time.

Table 2. Summary of Western Pacific Region's national laboratories performance

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Three countries reported using DBS (Cambodia, Fiji and the Philippines) and reported difficulties in obtaining sufficient samples volume. Only New Zealand reported using oral fluid samples for under 5-year-old cases.

Cambodia reported they carried out a measles and rubella serosurvey in women aged 15–39 years and found high immunity to measles (average 94.6%, range 92.0–97.2%) and lower immunity to rubella (average 74.4%, range 72.2–78.3%). Age group of the lowest immunity was 25–29 years for measles and 30–34 years for rubella. The Republic of Korea is planning to carry out a seroprevalence investigation for measles mumps and rubella in normal population aged 0–50 years.
Three national laboratories were establishing external quality assessment (EQA) programmes for subnational or private laboratories providing measles testing. The Republic of Korea developed an EQA for both serology and molecular testing for their provincial health laboratories and private diagnostic centres. Malaysia monitors the quality of the subnational laboratory established in 2011 in Sabah through EQA and confirmatory testing. New Zealand and Singapore reported that their non-network measles testing laboratories are accredited through ISO 15189 or CAP and may undergo EQA but positive samples are not confirmed at the national laboratory. Measles is notifiable in both countries.

The challenges in dealing with large measles outbreaks in Papua New Guinea, the Philippines and Viet Nam were compounded by surveillance not using EPI-links and sending samples from all suspected cases to the laboratory. The Philippines received 18 000 samples in the first two months of the outbreak but still managed to test 40% of these. Storage of samples was also a major problem but a new plan for improved national surge capacity with expanded testing capacity has been developed in collaboration with the Department of Health. Discussions have been held with surveillance and the EPI programme to facilitate utilizing epidemiological linkage. CRS surveillance (sentinel) was reported by five countries: China, Japan, Papua New Guinea, Singapore and Viet Nam.

### 2.2.8 Rubella global update

Only about 10% of countries reporting rubella cases are reporting genotype data and there is a critical need to improve this as determining baseline genetic information will be necessary for the verification process. The Region of the Americas has just been verified as having eliminated rubella. Three countries have excellent data on national rubella molecular epidemiology – China, Romania and Uganda. Uganda recently published evidence of co-circulation of two genotypes (1E and 1G) with three lineages of 1G over six years. The importance of CRS surveillance was described as early detection and intervention can reduce the long-term health consequences for infants. CRS surveillance is a challenge as around 50% of infections in pregnant women are asymptomatic, infection in pregnant women (first trimester) and its consequences (CRS in newborns) are separated in time, some defects (e.g. hearing impairment) require specialists or special equipment that may not be available, doctors may have insufficient experience to diagnose CRS, variable presentation depending on when maternal infection occurred in relationship to the gestational age and some infants only have one defect. A new guide is being developed for introducing rubella vaccine into national immunization programmes. The guide also covers CRS surveillance.

### 2.2.9 Rubella and CRS surveillance in the Western Pacific Region

**China**

Rubella surveillance has been operational since 1991. There are epidemic rubella cycles in China about every seven to eight years with outbreaks detected from 1993 to 1994, 2001 and 2008. A rubella containing vaccine (RCV) was introduced into EPI in 2008 and in 2014, a record low of 11 887 rubella cases (0.88/100 000) were reported and 19 of 31 provinces were found with incidence of less than 1/100 000. From 1991 to 2011 age groups of reported cases has increased. In 1991, 98% of cases were under 14 years and in 2011, 13% of cases were under 14 years.

Rubella molecular surveillance has been carried out since 1991 but integrated with measles surveillance in China in 2014. 1E genotype has been predominant since 2001. 2B genotype has co-circulated with 1E since 2006 but sporadically until 2010. Lineage 3 of 2B was introduced in 2011 and has become endemic in China. In 2015 (May) only 2B has been found.

CRS surveillance was established in 2009 as a special project in four cities in two provinces, Heilongjiang and Shandong. From 2009 to 2013, a total of 1670 suspected CRS cases were reported from Heilongjiang (661 cases) and Shandong (1009 cases). Of these, five from Heilongjiang province were laboratory-confirmed CRS cases, 59 were clinical-diagnosed CRS cases and two were identified as CRS cases. The challenges for CRS surveillance in China include difficulty in finding pregnant
women with rubella infection and difficulty in following up CRS cases and collecting specimens from these cases.

**Japan**

Rubella and CRS are notifiable in Japan. A large rubella outbreak occurred in Japan in 2013 with 14,357 cases detected. Most cases (N=10,985) were found in adult males (15–55 years), however 3,372 females were affected with the child-bearing age group (15-40 years) making up the majority of cases. A rubella antibody serosurveillance in 2014 identified adult females with high immunity across most ages but some gaps were found in people 20–40 years. Following the 2013 rubella outbreak, 40 CRS cases were reported. The outbreak diminished in 2014 with 321 cases detected and by May 2015, 70 cases were reported. The Japan Ministry of Health, Labour and Welfare has established a goal of CRS elimination as soon as possible and rubella elimination by 2020. Strategies to achieve this include strengthening immunization activities and improving surveillance. Virological surveillance has determined the 2013 outbreak was predominantly 2B (two strains) with a small number of 1E detected.

**Malaysia**

In Malaysia, rubella and CRS are not notifiable, however, rubella surveillance has been combined with measles surveillance since 2010 and a CRS diagnostic system has been established in the Institute of Medical Research. An online notification system for rubella/CRS has been established and data is shared monthly with WHO Regional Office for the Western Pacific. Rubella cases increased from 2011 to 2013 partly because of enhanced laboratory-based surveillance and EPI-linkages of cases. The age distribution of cases shows a peak in people 11–35 years, although more males (68–91%) than females are infected.

Measles, mumps, and rubella (MMR) vaccination was provided to both sexes from 2002. Prior to this, only adolescent girls and women of child bearing age were given monovalent rubella vaccine. Rubella viral surveillance has identified genotype information for the following years: 2012, 2B; 2013, 2B and 1E; 2014, 1A, 2B and 1E.

**2.2.10 Measles and rubella recommendations from the Fourth Meeting on VPD LabNet in the Western Pacific Region**

A total of 37 measles- and rubella-specific recommendations arose from the regional VPD LabNet meeting in March 2013. Almost all were addressed by the LabNet (97%) except for one that was related to the quality of DBS samples. Most of the partially implemented recommendations (38%) were related to testing strategies for samples collected in low-incidence settings when positive predictive value (PPV) is low. Most countries have yet to reach this stage due to numerous imported viruses being detected in the Region.

A summary of the accreditation status of the measles and rubella LabNet showed that almost all laboratories met the minimum criteria except for the timeliness of reporting within four days. The Philippines and Viet Nam laboratories dealt with large outbreaks in their countries and the logistics in testing and reporting most samples within four days after receipt were very challenging. Both countries also had test kit stockouts that impacted testing turnaround time. EPI-linkage was not implemented for the Philippines. Papua New Guinea experienced a large outbreak (more than 4,500 samples tested) but still managed to report most (89%) within seven days. One laboratory has pending accreditation due to confirmatory results being pending at the time of the review.

**2.2.11 Update on quality assurance for molecular proficiency testing**

In March 2014 a molecular PT was distributed to 22 reference laboratories in the LabNet. One laboratory had a problem and required a repeat test, which was passed. The global LabNet meeting agreed to an annual distribution of the molecular PT with distribution of the next panel in the third or fourth quarter of 2015. All national laboratories and regional reference laboratories will ultimately be
included. However, scale-up challenges will not allow all national laboratories to participate immediately. A commercial vendor (INSTAND) and US CDC will produce the panels and FTA cards will be used to stabilize the samples for ambient shipment and ensure they are non-infectious.

2.2.12 Measles and rubella IgM PT panel for 2014

The Western Pacific Region had 53 laboratories that participated in the 2014 global measles and rubella PT. Siemens was the most commonly used kits in the Region for both measles and rubella. In China, Haitai and Virion/Serion were mostly used for measles and rubella with Kerunda also used for rubella. Sample 17 and 19 were problematic in the Western Pacific Region for both measles and rubella for laboratories using Siemens. Both samples were negative in the reference laboratories but optical density (ODs) were slightly over cut off and weakly positive or equivocal in 18 laboratories for sample 17 and 13 laboratories for sample 19. These samples were excluded in the final analysis for those laboratories.

All laboratories in the Region passed PT although one laboratory had an invalid test. Two laboratories did not test the panel within the required 14 days and a further four laboratories did not report within 14 days. There is a plan to implement web-based submission of results for the next PT. The 2015 panel will be distributed at the time of the global meeting (June) for five regions and in August for the Western Pacific Region.

VIDRL supports the confirmatory testing for seven national laboratories in the Region (Brunei Darussalam, Fiji, Guam, Malaysia, New Zealand, Papua New Guinea and the Republic of Korea). All national laboratories were found to have acceptable concordance (more than 90%) for both measles and rubella rest results with the reference laboratory.

2.2.13 Confirmatory testing by Hong Kong SAR (China) RRL

The Hong Kong SAR (China) RRL supports eight national laboratories (Cambodia, the Lao Democratic Republic, Macao SAR (China), Mongolia, the Philippines, Singapore and Viet Nam (Hanoi and Ho Chi Minh City)) for confirmatory testing. In 2014, 1314 serum samples were received for measles and rubella and 290 were selected for RT-PCR testing for molecular surveillance purposes. All national laboratories achieved concordance scores of more than 90%.

It was considered that WHO is not using a definitive method for defining discordance when qualitative results are discordant but quantitative results are similar. The Hong Kong SAR (China) laboratory uses a cut off of more than 0.1 ODs to define discordance between two results of the same sample. However this can only be done when the same assay is used, and for Siemens, the correction factor is used. A selection of samples are also tested by nested RT-PCR for molecular surveillance purposes if samples are collected within five days of onset, are IgM positive and have been transported by reverse cold-chain. It was suggested by the RRL that a schedule of testing be arranged by the Regional Laboratory Coordinator to ensure that large batches of samples do not arrive at the same time at the RRL nor do they arrive over periods when the laboratory is closed.

2.2.14 China LabNet confirmatory testing and EQA for provincial laboratories

The National Measles and Rubella Laboratory for China at the China Center for Disease Control and Prevention (CDC) provide quality assurance for the 31 provincial laboratories in the country. The global PTs for 2013 and 2014 were distributed and the results showed all laboratories achieved 100% in 2013 and all with 100% in 2014 except two, which achieved 95%. Confirmatory testing of all the provincial laboratories by the national laboratory resulted in concordance scores of more than 95% for measles and >90% for rubella. Accreditation reviews in China are carried out annually for five to six provincial laboratories and the national laboratory by international experts in combination with national laboratory staff. Some of the issues identified by the reviewers were: some standard operating procedures (SOPs) are outdated and need revising; provincial laboratory staff to work with EPI staff to get more representative virological strains; proper directional workflow for molecular testing
needed; provincial laboratories to monitor the quality of the prefecture and county level laboratories; and utilization of FTA cards should be considered for safe shipment within China.

### 2.2.15 Reporting measles laboratory data to WHO and introduction of MR surveillance reporting system (MRSRS)

The regional measles and rubella LabNet data is collected for four major reasons: reporting monthly to WHO headquarters; publishing in the WHO regional measles bulletin; providing data to the Health Information and Intelligence Platform website; and updating the measles/rubella country profile data. Laboratories submit data monthly in a variety of formats. Some core variables are not being reported and different formats and codes are used. Typographical errors also occur and data cannot be linked to surveillance data.

MRSRS is a web-based system designed to contain data from both surveillance and laboratory. It has the same basic feature as the access database but with the advantages that it is automatically linked to surveillance data; data are instantly reported; and multiple users can enter data at the same time. It is currently being used by Cambodia (since 2013), the Lao People's Democratic Republic (since 2014) and is being tested by Viet Nam.

In 2010, the Cambodia National Laboratory was using the old Access Database for measles/rubella reporting. The issues with this database included: no systematic way to back up; updating the database was complex and needed WHO Regional Office for the Western Pacific support; separate databases for laboratory and surveillance which were not linked and datasets were different; and data reports were not in line with new programme indicators. In 2013 the new database started to be used and made significant improvements for measles/rubella surveillance in the country. This included: timely reporting of laboratory results; ability to review provincial level surveillance performance and provide rapid feedback; allowed several staff to access core data set, especially during field visits; the possibility of complete integration and match of the surveillance and laboratory data.

### 2.2.16 Roundtable sessions: strengthening laboratory management

Participants were divided into four discussion groups:

1) ELISA IgM testing: Discussion points included: correct use and monitoring of in-house controls (IHC) to detect random and systematic error; use of the Levey-Jennings control chart, quality control problems due to uncalibrated equipment, poor technique; freeze aliquots at -20°C to maintain IgM stability; plot values from runs of five controls done in three consecutive days; follow WHO protocol on IHC preparation. Outbreak management issues include: training more staff to increase surge capacity; encourage EPI-linking strategy to reduce workload of IgM testing/genotyping; make a buffer number of kits to alert when to order; and regularly check inventory sheets.

2) Virus isolation and molecular detection: discussion points included: representative isolates are needed to use expanded sequence windows or whole genome sequencing; no problems with recovery of cells; do we need to introduce susceptibility testing after recovery and if so do we need a standard protocol?; mycoplasma testing should be standardized with recommended PCR protocol provided; SOPs for clean workflow needed; contamination from samples can be combated with filtration only on samples that show contamination on initial test; throat swabs are preferred over urine and should be collected within five days for virus isolation; choosing representative samples for isolation and expanded molecular analysis is needed.

3) Data management and reporting: data management and reporting to the sequence databases were discussed. Conclusions were: an agreement that the naming of sequences requires three administrative levels for the location – city, province and country; the use of “named strains” is critical to identify linkages between countries and regions; having separate databases promotes discordance between the databases and countries are encouraged to report systematically to only MeaNS and the WHO Regional Office for the Western Pacific uses MeaNS for all data analysis; MRSRS could be expanded
to include more MeaNS and RubeNS data elements to connect the databases; multiple sequences take too long to load however there is an option to batch upload provided by Richard Myer of Public Health England.

4) Communication and collaboration: discussion points included: communication and collaboration with RRLs and the WHO Regional Office for the Western Pacific is critical; need to coordinate schedule and make up of samples for the confirmatory tests by RRLs (Hong Kong, VIDRL) and national laboratories (NLs); China CDC is responsible for China LabNet’s provincial and prefectural laboratories; coordination of test kit procurement; and emergency response may need support from the WHO Regional Office stocks. To build capacity: workshops and training for subnational laboratories are being considered for the Philippines and Viet Nam; capitalize on existing subnational facilities for other diseases (e.g. flu, dengue); relationship between private laboratory and national laboratory requires coordination by the NL with the private laboratories to introduce a QA programme, data sharing; and suggestions that WHO provide a framework to countries for sharing data from private laboratories.

2.3 Poliovirus biorisk management training

The *Polio Eradication and Endgame Strategic Plan 2013–2018* set the goal of a polio-free world by 2018. Following global type-specific interruption of WPV transmission and sequential cessation of OPV use, safe handling and biorisk management of poliovirus infectious and potentially infectious materials will be essential to minimize the risk of reintroducing the virus into the community. The third edition of GAP III aligns the safe handling and containment of poliovirus infectious and potentially infectious materials with the endgame strategy. GAP III describes timelines and requirements for laboratories and addresses type-specific containment of WPV as well as OPV/Sabin polioviruses. It establishes the long-term goal of minimizing the risk of facility-associated poliomyelitis in the post-eradication/post-bOPV era by providing continued access to safe and affordable IPV or Sabin-IPV and by reducing to a minimum the number of facilities handling and storing polioviruses while serving essential functions and meeting all required safeguards.

Most countries will have no need to retain live polioviruses in the post-eradication and post-OPV era. Facility-associated risks in these countries can be eliminated by a thorough nationwide search for and destruction of all WPV and all OPV/Sabin infectious and potentially infectious materials. Some countries will host a limited number of poliovirus facilities that serve critical international functions, including IPV and Sabin-IPV production, production and storage of mOPV stockpiles, vaccine quality assurance, diagnostic reagent production, virus diagnostic and reference functions, together with crucial research. Each of these facilities should manage biorisk appropriately to minimize the risk of virus reintroduction into the community, with effective national certification and WHO verification programmes.

Biorisk management in designated essential poliovirus facilities will be achieved through the implementation of international biorisk management standards that: 1) include polio-specific containment requirements to reduce the likelihood of release of polioviruses from essential poliovirus facilities (primary safeguards); 2) describe population immunity requirements (secondary safeguards) to minimize the consequences of the release of polioviruses from essential poliovirus facilities; and 3) define the site-specific environmental requirements for essential poliovirus facilities (tertiary safeguards) to minimize the consequences of potential release.

Implementation of GAP III calls for training in biorisk management for handling and storing WPV2 of various stakeholders in the Western Pacific Region. The first poliovirus biorisk management training was organized for members of Regional Polio Laboratory Network. The training aimed to provide the participants with an understanding of the background to GAP III including the current status of the eradication programme, general GAP III requirements and how GAP III needs to be implemented, its potential impact and timelines. Participants were introduced to the biosafety and biosecurity management system principles and concepts as stated in GAP III.
3. CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

3.1.1 Polio

The meeting concluded that the performance of the regional polio laboratory network has been sustained at polio-free-certification standard and that AFP surveillance activities have been efficiently supported. The network laboratories provided critical evidence in support of the continued polio-free status of the Region. As of March 2015, all 43 network laboratories are fully accredited including all 33 polio laboratories with ITD function. All ITD laboratories passed the ITD PT, and 22 ITD laboratories scored 100%. Six ITD laboratories also participated in the polio sequencing PT, and four ITD laboratories scored 100%. Seven laboratories are planning to participate in the polio sequencing PT in 2015.

3.1.2 Measles and Rubella

The meeting concluded that measles and rubella network laboratories have helped in working towards the regional goal of measles and rubella elimination. The laboratories have confirmed suspected cases and identified measles and rubella virus genotypes circulating in the Region. The LabNet has played a critical role in the recent verification of measles elimination of seven Member States by confirming that measles cases found in these countries are imported rather than due to endemic circulation. The network consists of one global specialized laboratory in Japan, three regional reference laboratories in Australia, China and Hong Kong SAR (China), 14 fully functional national measles and rubella laboratories, 31 provincial and 331 prefectural laboratories in China, and three new subnational laboratories in Malaysia and Viet Nam. Among 49 laboratories for which WHO conducted on-site reviews for accreditation, 48 laboratories are fully accredited as of March 2015. One laboratory with pending accreditation status needs to complete the confirmatory testing.

3.1.3 Poliovirus biorisk management training

The first poliovirus biorisk management training provided participants with an understanding of the background to GAP III laboratory requirements and how these will need to be implemented, together with potential timelines. Participants were introduced to the physical containment and biorisk management system requirements (the latter being based upon the CWA 15793; Laboratory Biorisk Management standard) and associated biosafety and biosecurity management system principles and concepts in line with GAP III needs. The training also provided clarification as to how the associated assessment and certification mechanisms of essential laboratories may function and to prepare participants to overcome challenges that may be encountered during implementation of GAP III.

3.2 Recommendations

3.2.1 Polio

1) As specified in the International Health Regulations (2005) (IHR) all non-Sabin polioviruses, including VDPV and WPV, should be reported to WHO within 24 hours of confirmation. All type 2 strains should be considered as non-Sabin three months after the switch to bivalent OPV. Participants are reminded of the definition of VDPV: types 1 and 3 require 10 or more nucleotide changes and type 2 requires six or more nucleotide changes.

2) AFP results should be reported to WHO Regional Office for the Western Pacific weekly (every Friday). Zero reporting should be used when no AFP cases are detected. The reporting of polioviruses from non-AFP surveillance data – except environmental surveillance – should be shared monthly in an aggregated format.

3) Environmental surveillance: reporting to the Regional Office should adhere to the timeframe outlined by the Technical Advisory Group (TAG) 2014. It is recommended that countries
conducting environmental surveillance identify and characterize polioviruses using WHO-recommended methods and that the results be shared with the Regional Office at least on a weekly basis.

4) Laboratories are recommended to maintain the recommended maximum passage number cut off of 245 for rhabdomyosarcoma (RD) and 35 for mouse cell line that express the gene for the human cellular receptor for polioviruses (L20B). New low passage cells are being considered for use in the Global Polio Laboratory Network (GPLN) and will be utilized to extend the useable life span of these cells. The new low passage cells will be distributed to GPLN when available.

5) Introduction of the new US CDC version 4.0 ITD method is planned for early 2015 in the Region. Laboratories should continue to use ITD version 3.0 until ITD version 4.0 has been received. Laboratories should determine any need for primers and other reagents and report these to the RLC.

6) Most countries have developed a polio outbreak response plan. Laboratory directors are encouraged to ensure the plan includes a component that deals with the implications for the laboratory during an outbreak, especially related to surge capacity, and should include consideration for increasing staff, equipment and consumables during periods of heavy workload.

7) Containment issues related to OPV type 2 following the introduction of bivalent OPV planned for April 2016 will affect the GPLN. The introduction will especially impact the continuation of: cell sensitivity tests; viral isolation proficiency tests; and serum neutralization assays after the switch. The necessity for countries to carry out seroprevalence studies should follow discussions held at national, regional and global levels.

8) ITD laboratories with challenges in shipping isolates or RT-PCR products to sequencing laboratories for further investigation are encouraged to use FTA cards and follow the recommended GPLN protocol to ensure samples are rendered non-infectious before shipment.

9) The new ES draft guidelines are now available and all participants should review and share their comments with WHO headquarters by the end of June. The guidelines are available on the USB files provided with the meeting presentations.

10) The evidence that L20B contains mouse endogenous retrovirus reminds GPLN that they need to consider all cell culture material as potentially infectious.

11) Laboratories should be aware of viruses growing in cell lines which do not follow normal patterns. Detection of non-polio virus growing in L20B should be further investigated and cell line authentication considered using validated protocols recommended by the GPLN. All regional cell repositories – NIID, VIDRL and China CDC – should only distribute validated cell lines that have been tested by a recognized “ authenticating laboratory”.

12) Mycoplasma testing of stock cells and working cells should be undertaken regularly. Cell stocks should be tested soon after they have been frozen and routine working stocks should be checked near the end of the 15 recommended passages to maximize the chances of detecting mycoplasma contamination. If a laboratory detects mycoplasma then the frequency of testing should be increased.

13) All countries in the Region should complete type specific poliovirus inventories in 2015, nominate a national containment coordinator and identify a national regulatory agency for containment by the end of June 2015.

14) It is recommended that laboratories use the new GPLNMS and upload the information requested, including: laboratory information, staff, equipment, publications and proficiency testing. Data should be submitted to the RLC at the earliest convenience of each laboratory.
15) All laboratories are encouraged to use the Western Pacific Region Access database to share laboratory data with the Regional Office according to the prescribed timeliness indicators. The Regional Office is developing a new Polio AFP Surveillance Reporting System (PASRS) web-based database. The database should be ready for implementation in the last quarter of 2015. Laboratories will be notified when the database is ready to be used and are encouraged to use the Beta version.

16) Many laboratories in the Region are detecting very few polioviruses in routine AFP testing. It is recommended that laboratories use virus stock from their sensitivity testing to maintain expertise in polio isolation and detection.

3.2.2 Measles and rubella

A. General

1) National laboratories that have responsibility for subnational laboratories should carefully monitor their performance including performing confirmatory testing, proficiency testing and kit validation if subnational laboratory (SNL) is not using a validated WHO recognized kit.

2) Laboratories that are routinely performing molecular testing for measles and rubella should enrol in the WHO/US CDC molecular External Quality Assessment (mEQA) programme to assess their performance as part of the annual accreditation of the laboratory. The global molecular PT panel will be expanded in 2015 and Western Pacific Region laboratories are encouraged to participate.

3) All network laboratories are required to participate in the annual accreditation programme for measles and rubella laboratories either by correspondence or in conjunction with an on-site visit by the WHO RLC or her/his representative.

4) The WHO Regional Office for the Western Pacific has established a four-day turnaround time for reporting IgM results after receipt (more than 80%). Laboratories in countries experiencing large outbreaks should not be penalized for not meeting the four-day turnaround but should communicate any timeliness issues to the RLC as soon as possible.

5) Some countries are preparing molecular PT panels for their subnational laboratory networks. It would be beneficial to the global programme if information on the composition and results from these panels is shared with WHO Regional Office for the Western Pacific and WHO headquarters.

6) Countries needing positive serum samples for quality control should communicate with the RLC.

7) Confirmatory testing has become a critical tool to monitor the performance of LabNet laboratories. All laboratories are requested to participate. For better classification of concordance, it is suggested:
   a. to develop a standardized protocol to define concordance among different RRLs; and
   b. to include corrected OD in the sample lists.

8) Regular communication and coordination between the RLC, national laboratory and RRL is important to facilitate the process of confirmatory testing:
a. send a draft sample list to RLC and RRL before shipment for confirmation (approximately 10% or minimum of 15, priority order: positives, equivocal, negatives);

b. arrange scheduled shipment of specimens; and

c. national laboratories are suggested to provide feedback to RRL and the RLC for the follow up of discordant results.

9) The use of an IHC is a proven tool to monitor assay performance. Laboratories should include an IHC with every test where possible. Laboratories should use Westgard plots to monitor assay performance. In countries with a lack of acute measles and rubella cases, the RRLs are requested to assist in the provision of IHC.

B. Measles

1) Laboratories in the Western Pacific Region perform virological surveillance to help measure progress towards measles elimination and/or to verify measles elimination. The reporting of virological surveillance data is very important for national and regional verification committees. To improve the quality of reporting of viral surveillance, laboratories should:

   a. perform phylogenetic analysis of the sequences from all cases that have been genotyped;

   b. attempt to match the sequences to a named lineage in MeaNS to help identify transmission chains and potential sources of importation;

   c. attempt to obtain genetic information from at least 80% of chains of transmission and include the percentage of chains of transmission with genotype in the national report;

   d. share sequence data globally so timely submission of sequences to MeaNS is critical. Laboratories should become familiar with MeaNS and be aware of the concept of the utility of named strains. Laboratories are reminded that timeliness of sequence submission is a performance indicator as defined in the WHO accreditation checklist; and

   e. LabNet should develop additional guidance on the diagnostic implications of accepting respiratory or urine specimens as adequate for surveillance purposes.

2) Use of expanded sequence windows and whole genome sequencing will require representative viral isolates. The Region's LabNet is encouraged to support viral isolation at all RRLs and several national laboratories (NLs). Laboratories that do not have capacity for virus isolation may be asked to refer clinical samples for virus isolation so that these isolates can be used for expanded sequencing.

3) Regional reference laboratories should consider testing IgM positive serum samples for the presence of viral ribonucleic acid (RNA) to expand the genetic databases for measles and rubella (however sera should not be to the exclusion of better samples such as throat swabs or virus isolates). To enhance the success rate from serum samples sent for confirmatory testing, it is suggested:

   a. ensure optimal condition of specimens during transport, dry ice is preferred but ice pack can be considered if the shipment time is short. It is suggested to freeze the specimen before shipment if an ice pack is used;

   b. include the onset date and specimen collection date in the sample list;

   c. send non-serum samples such as respiratory specimens for genotyping; and

   d. ensure a sufficient volume of serum sent (at least 200ul serum) to RRLs.
4) The LabNet is urged to obtain sequence information from all outbreaks and chains of transmission according to WHO guidelines with a minimum of 80%. Appropriate clinical specimens should be collected. The national surveillance programmes are urged to collect appropriate specimens for molecular analysis. The laboratories can provide guidance on sample collection, transportation and storage.

5) An update to measles viral surveillance is planned for weekly epidemiological report (WER) in early July. Laboratories are invited to give timely feedback on the draft WER through the RLC.

6) Laboratories should work closely with their national verification committees to ensure data are properly represented in annual national reports to the Regional Verification Commission.

7) Laboratories are commended in their efforts to isolate measles and rubella virus strains on cell cultures. These laboratories are kindly requested to submit representative isolates to WHO's measles and rubella virus strain bank.

8) Countries experiencing large outbreaks should follow the surveillance guidelines and use the mechanisms for EPI-linking cases after each chain of transmission has been laboratory-confirmed.

9) National laboratories should ensure their national surveillance/outbreak response plans include a contingency plan for sampling strategies in the event of a large outbreak to ensure the number of samples collected do not overwhelm the capacity of the laboratory. Once the outbreak has been confirmed, sample collection should focus on areas of the country which are newly infected, high risk, and any other new chains of infection. EPI-linkage case confirmation, in conjunction with the surveillance programme, should be fully utilized.

C. Rubella

1) If CRS is suspected after six months and infant samples are IgM negative, rubella IgG ELISA may help in case classification if the infant is not vaccinated and has no history of exposure to wild-type viruses. However, in endemic areas, IgG testing may not be useful for case classification of CRS. RRLs and GSLs should provide guidance on protocols for testing IgG to confirm CRS.

2) Samples from CRS patients are a source for virus isolation and genotyping. Almost all CRS infants excrete virus at birth and some continue to excrete virus for months after birth. After four months, the proportion of CRS infants shedding virus decreases to 50–60%. For molecular testing, the optimal specimens from suspected CRS cases are nasopharyngeal secretions and urine (cataracts are not optimal).

3) Molecular surveillance: The network laboratories should continue making full efforts to obtain complete genotype and sequence information on measles and rubella viruses circulating in the Region using the proper molecular window. Laboratories should work in collaboration with the epidemiology group to differentiate imported cases from endemic cases. Molecular surveillance should emphasize isolates for rubella if possible, including stored isolates from the last three years.

4) Virological surveillance is a critical component of laboratory surveillance for rubella. There is a critical need to expand the database of viral sequences from the Region and especially for rubella. Laboratories are recommended to submit sequence information for rubella viruses to RubeNS, as soon as it is available.
5) All laboratories should develop an algorithm for testing samples from suspected cases of measles, rubella and CRS. This algorithm should be developed along with national epidemiologists and include the use of available laboratory methods for case classification. This algorithm should include a plan for referral of samples as necessary to RRLs and GSLs for tests that are not available at the national level (e.g. IgG avidity).

6) Considering the rubella and CRS elimination initiative, network laboratories should be prepared to support laboratory surveillance for rubella and CRS and receive test samples from suspected rubella and CRS cases. RRLs and GSLs should develop guidance to assist laboratories in preparing to move towards rubella (and CRS) enhanced control and elimination.

7) Testing for the presence of rubella IgM in pregnant women when there has been no evidence of rubella infection or contact with rubella cases is not recommended. Such testing is likely to produce false-positive results in approximately 1% of those tested, as the specificity of the Siemens assay is approximately 98–99%. Rubella IgG testing can be used for screening of asymptomatic pregnant woman to look for rubella immunity. Non-immune women should be vaccinated against rubella after completion of the pregnancy.

8) In consultation with epidemiologist/public health colleagues at national and regional levels, laboratories should develop a system for assessing and reporting virus shedding (contagiousness) for confirmed CRS cases.

3.2.3 Recommendations for WHO Secretariat

1) Due to resource constraints, polio and measles/rubella laboratory accreditation by correspondence will be encouraged. Laboratories to undergo on-site review will be those in priority countries and/or those with challenges in meeting quality assurance and quality control performance levels and those that have not undergone an on-site review for three or more years.

2) The new web-based measles and rubella database (MRSRS) being proposed for the Region has considerable potential for enhancing the exchange of data between the laboratory and the Regional Office. The potential to expand this platform for other purposes, such as CRS surveillance, should be considered once MRSRS is fully operational.

3) Future training should include appropriate training for laboratory aspects of CRS surveillance.

4) The Regional Laboratory Coordinator (RLC) should determine ITD version 4.0 training requirements and respond as appropriate. The RLC should update the transition plan and share with WHO headquarters and US CDC.
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### PROVISIONAL TIMETABLE

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<td>• Self-introduction</td>
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<td>• Election of officers: Chairperson, Vice-Chairperson and Rapporteur</td>
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<td>• Administrative announcements</td>
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<tr>
<td>09:00 – 09:10</td>
<td>GROUP PHOTO</td>
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<tr>
<td>09:10–10:10</td>
<td>Session 1. Polio Endgame Strategy and updates on maintaining polio-free status: Global and Regional</td>
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<tr>
<td></td>
<td>a) Polio endgame strategy and WPRO update on the polio eradication initiative and next steps</td>
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<td></td>
<td>b) Update of Global WPV transmission and status of polio laboratory network</td>
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<td></td>
<td>c) Regional update of polio laboratory network</td>
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<td>10:10–10:30</td>
<td>COFFEE BREAK</td>
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<td>10:30–11:30</td>
<td>Session 2. Vaccine-derived poliomyelitis virus (VDPV)</td>
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<tr>
<td></td>
<td>a) Methodologies for VDPV detection, characterization and virologic classification</td>
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<td>b) VDPV surveillance in the Philippines</td>
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<td>c) VDPV surveillance in China</td>
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<tr>
<td></td>
<td>Discussion</td>
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<tr>
<td>11:30–12:30</td>
<td>Session 3. Reports from polio laboratories</td>
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<tr>
<td></td>
<td>a) Australia</td>
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<td>c) Japan</td>
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<td>d) Hong Kong SAR (China)</td>
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<td>12:30–13:30</td>
<td>LUNCH BREAK</td>
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</tbody>
</table>

**Session 5. Update on diagnostics and new technologies**

- a) Cell line authentication
- b) Introduction of FTA card for shipment of cell lysate: VIDRL experience
- c) Update on the new ITD algorithm and CDC rRT-PCR assays ver. 4.0 for ITD and VDPV screening

**Session 6. Detection of poliovirus from non-AFP specimens and environmental surveillance**

- a) Global perspectives on environmental surveillance and plans for expansion
- b) Environmental surveillance of poliovirus and non-polio enteroviruses
  - Australia
  - China
  - Malaysia
  - Japan

**Session 7. Experience of polio laboratory network for the laboratory diagnosis of hand, foot and mouth disease (HFMD) and other enteroviruses**

- a) HFMD in China
- b) HFMD in Japan
- c) HFMD in Viet Nam
<table>
<thead>
<tr>
<th>Time</th>
<th>Monday, 25 May 2015</th>
<th>Time</th>
<th>Tuesday, 26 May 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:30–15:00</td>
<td><strong>Session 3. Reports from polio laboratories — continued</strong></td>
<td>13:15–14:00</td>
<td><strong>Session 8. Data management and polio laboratory database</strong></td>
</tr>
<tr>
<td>13:45–14:00</td>
<td>f) Mongolia</td>
<td>13:30–13:45</td>
<td>b) Update on implementation of Regional database distributed in 2013</td>
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<tr>
<td>14:00–14:15</td>
<td>g) New Zealand</td>
<td>13:45–14:00</td>
<td>Discussion</td>
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<td>14:15–14:30</td>
<td>h) Philippines</td>
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<td>14:30–14:45</td>
<td>i) Republic of Korea</td>
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<td>14:45–15:00</td>
<td>j) Singapore</td>
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<td>15:00–15:30</td>
<td><strong>COFFEE BREAK</strong></td>
<td>15:30–16:00</td>
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<tr>
<td>15:30–16:30</td>
<td><strong>Session 3. Report from polio laboratories — continued</strong></td>
<td>15:30–16:00</td>
<td><strong>Session 9: Group discussion on laboratory management issues</strong></td>
</tr>
<tr>
<td>15:30–15:45</td>
<td>a) Viet Nam – North</td>
<td></td>
<td>Group 1: Contingency planning in WPR polio laboratories post eradication</td>
</tr>
<tr>
<td>15:45–16:00</td>
<td>b) Viet Nam – South</td>
<td></td>
<td>Group 2: Data management and timeliness of reporting</td>
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<tr>
<td>16:00–16:10</td>
<td>Discussion</td>
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<td>Group 3: Quality Assurance and Quality Control</td>
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<td>16:00–16:30</td>
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<td>16:30–17:30</td>
<td><strong>Session 4. Laboratory Quality Assurance</strong></td>
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<tr>
<td>16:30–16:45</td>
<td>a) Follow-up on recommendations from 2013 Regional Polio Laboratory Network Meeting</td>
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<tr>
<td>16:45–17:00</td>
<td>and 2014/2015 accreditation status</td>
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<td>17:00–17:15</td>
<td>b) Report on 2014 virus isolation proficiency testing</td>
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<td>17:15–17:45</td>
<td>c) Update on new scoring pattern and report on 2014 ITD and sequencing PT</td>
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<td>17:45–18:00</td>
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<td>16:30–17:30</td>
<td>Session 10. Update on other VPD surveillance and laboratory networks in the Region</td>
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<td>16:30–17:30</td>
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<tr>
<td>17:30–17:45</td>
<td><strong>Session 11. Conclusions and recommendations</strong></td>
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<td></td>
<td>Closing of the meeting</td>
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</table>
### Provisional Timetable

#### Part III: Measles and Rubella Laboratory Network Meeting, 27–28 May 2015

<table>
<thead>
<tr>
<th>Time</th>
<th>Wednesday, 27 May 2015</th>
<th>Thursday, 28 May 2015</th>
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</thead>
<tbody>
<tr>
<td>08:00–08:30</td>
<td>Registration</td>
<td>08:30–10:00</td>
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<tr>
<td>08:30–09:00</td>
<td>Opening session</td>
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<tr>
<td></td>
<td>• Opening remarks</td>
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<td>• Self-introduction</td>
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<td></td>
<td>• Election of officers: Chairperson, Vice-Chairperson and Rapporteur</td>
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<td>• Administrative announcements</td>
<td>09:40–10:00</td>
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<td>09:00–09:10</td>
<td>GROUP PHOTO</td>
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<tr>
<td>09:10–09:15</td>
<td>• Meeting objectives and outcomes</td>
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<tr>
<td>09:15–10:10</td>
<td>Session 1. Overview of global and regional measles elimination and rubella control initiatives</td>
<td>10:00–10:30</td>
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<tr>
<td></td>
<td>a) Global and Regional updates on eliminating measles and rubella</td>
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<tr>
<td></td>
<td>b) Update of Global measles and rubella laboratory network</td>
<td>10:30–10:45</td>
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<td></td>
<td>c) Progress – Regional measles and rubella laboratory network</td>
<td>10:45–10:55</td>
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<td>10:30–11:30</td>
<td>Session 8: Strengthening Rubella and CRS Surveillance</td>
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<td>10:30–11:30</td>
<td>Session 2. Reports from GSL and RRLs</td>
<td>11:00–11:15</td>
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<td></td>
<td>a) Japan</td>
<td>10:30–11:30</td>
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<td>b) Australia</td>
<td>10:45–10:55</td>
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<td>c) China</td>
<td>11:05–11:15</td>
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<td></td>
<td>d) Hong Kong SAR (China)</td>
<td>11:15–11:30</td>
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<td>11:30–12:30</td>
<td>Session 3. Molecular surveillance</td>
<td>12:00–12:15</td>
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<tr>
<td>11:30–11:45</td>
<td>a) Global update on circulating measles genotypes</td>
<td>12:15–12:30</td>
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<td>11:45–12:00</td>
<td>b) Regional update on molecular surveillance of measles</td>
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<td>12:00–12:10</td>
<td>c) Discordance of genotype maps between WHO headquarters and WPRO, and MeaNS and RubeNS surveys</td>
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<td>12:10–12:30</td>
<td>Discussion</td>
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<td>12:30–13:30</td>
<td>LUNCH BREAK</td>
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<tr>
<td>Time</td>
<td>Wednesday, 27 May 2015</td>
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<td>Commission for Measles Elimination in WPR</td>
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<td>13:45–14:00</td>
<td>b) The role of LabNet to support verification of measles</td>
<td>13:45–14:00</td>
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<td>elimination</td>
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<td>14:00–14:20</td>
<td>Discussion</td>
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<td>14:15–14:30</td>
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<tr>
<td>14:20–15:05</td>
<td>Session 5: Country reports (Part I)</td>
<td>14:30–15:30</td>
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<td>14:20–14:35</td>
<td>a) Brunei Darussalam</td>
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<td>14:35–14:50</td>
<td>b) Cambodia</td>
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<td>14:50–15:05</td>
<td>c) Republic of Korea</td>
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<td>15:05–15:30</td>
<td><strong>COFFEE BREAK</strong></td>
<td>15:30–16:00</td>
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<tr>
<td>15:30–16:20</td>
<td>Session 5. Country reports (Part I) — continued</td>
<td>16:00–17:00</td>
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<tr>
<td>15:35–15:50</td>
<td>a) New Zealand</td>
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<td>15:50–16:05</td>
<td>b) Mongolia</td>
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<td>16:05–16:20</td>
<td>Discussion</td>
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<tr>
<td>16:20–17:35</td>
<td>Session 6. Country reports (Part II)</td>
<td>17:00–17:30</td>
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<tr>
<td>16:20–16:35</td>
<td>a) Malaysia NML and SNL</td>
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<td>16:35–16:50</td>
<td>b) Lao People's Democratic Republic</td>
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<td>16:50–17:05</td>
<td>c) Singapore</td>
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<td>17:05–17:20</td>
<td>d) Fiji and PICs</td>
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<td>17:20–17:35</td>
<td>Discussion</td>
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<tr>
<td>17:35–18:00</td>
<td>Wrap up and close of the first day</td>
<td>17:30</td>
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<td>18:30</td>
<td>Regional Director's Reception</td>
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</table>
GAPIII Implementation training for candidate essential poliovirus laboratory facilities and non-essential laboratory facilities that are likely to investigate, as of Phase II, new WPV2, aVDPV2, cVDPV2. Or iVDPV2 isolates, or new faecal or respiratory samples originating from recent OPV-using countries.

Course Objectives

This course aims to provide the participants with an understanding on:

- The background to GAPIII including the current status of the eradication programme and associated overview information
- How GAPIII needs to be implemented, its potential impact and timelines
- GAPIII requirements
- Biosafety and biosecurity management system principles and concepts for GAPIII
- How the associated assessment and certification mechanism may function

Pre-requisites

Another intention of this course is to collect feedback on the proposed measures and how these will impact the respective parties concerned. It is therefore recommended that the participants have a good understanding of the following background materials:

- GAPIII – WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use
  (http://www.polioeradication.org/Resourcelibrary/Posteradicationpolicydocuments.aspx);

Participants are also required to come prepared with:-

- Questions on GAPIII and any other related issues;
- Updated facility inventory list with information on type 2 polioviruses (WPV and Sabin).
# Course Schedule

**27 – 30 May 2015**

## Day 1 – GAP III Introduction - background and basis

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>0830</td>
<td>Registration</td>
</tr>
<tr>
<td>0900</td>
<td>Pre-course assessment, course objectives and introduction</td>
</tr>
<tr>
<td>1000</td>
<td>Break</td>
</tr>
<tr>
<td>1020</td>
<td>Overview of the current eradication programme &amp; status, the Endgame Plan and the switch</td>
</tr>
<tr>
<td>1200</td>
<td>Lunch</td>
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<tr>
<td>1300</td>
<td>GAPIII – Implementation expectations, implications and timelines</td>
</tr>
<tr>
<td>1500</td>
<td>Break</td>
</tr>
<tr>
<td>1520</td>
<td>Basis for biorisk management system with exercise</td>
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<tr>
<td>1730</td>
<td>Conclusions with Q &amp; A</td>
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</tbody>
</table>

## Day 2 – Scope and content of a biorisk management system – Elements 1 & 2

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>0900</td>
<td>Element 1: Biorisk Management System</td>
</tr>
<tr>
<td>1030</td>
<td>Break</td>
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<tr>
<td>1050</td>
<td>Element 1: Biorisk Management System</td>
</tr>
<tr>
<td>1200</td>
<td>Lunch</td>
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<tr>
<td>1300</td>
<td>Element 2: Risk Assessment</td>
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<tr>
<td>1500</td>
<td>Break</td>
</tr>
<tr>
<td>1520</td>
<td>Element 2: Risk Assessment</td>
</tr>
<tr>
<td>1645</td>
<td>Element 2: Risk Assessment</td>
</tr>
<tr>
<td>1700</td>
<td>Conclusions with Q &amp; A</td>
</tr>
</tbody>
</table>
Day 3 – Scope and content of a biorisk management system – Elements 3 - 11

0900 – 1030   Element 3: Poliovirus Inventory and Information and exercise
Element 4: General Safety

1030 – 1050   Break

1050 – 1200   Element 5: Personnel and Competency and exercise
Element 6: Good Microbiological Techniques

1200 – 1300   Lunch

1300 – 1500   Element 7: Clothing and Personal Protective Equipment (PPE) and exercise
Element 8: Human Factors

1500 – 1520   Break

1520 – 1645   Element 9: Healthcare
Element 10: Emergency Response and Contingency Planning and exercise
Element 11: Accident/Incident Investigation

1645 – 1700   Conclusions with Q & A

Day 4 – Scope and content of a biorisk management system – Elements 12 - 16

0900 – 1030   Element 12: Facility Physical Requirements

1030 – 1050   Break

1050 – 1200   Element 13: Equipment and Maintenance
Element 14: Decontamination, Disinfection and Sterilisation and exercise
Element 15: Transport Procedures
Element 16: Security and exercise

1200 – 1300   Lunch

1300 – 1400   Assessment against GAPIII requirements (internal & external)

1400 – 1530   Planning for implementation exercise

1530 – 1550   Break

1550 – 1620   Post-course assessment

1645 – 1700   Roundtable Q&A, feedback, conclusions and next steps