

## Hands-on Training Workshop on Cell Culture Techniques for the Laboratory Diagnosis of Polio/Enteroviruses and Measles/Rubella in the Western Pacific Region



19–23 May 2014  
Hong Kong (China)



**World Health  
Organization**

**Western Pacific Region**



Participants of the Hands-on Training Workshop on Cell Culture Techniques for the Laboratory Diagnosis of Polio/Enteroviruses and Measles/Rubella viruses in the Western Pacific Region, 19-23 May 2014, Hong Kong (China)

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**REPORT**

**HANDS-ON TRAINING WORKSHOP ON CELL CULTURE TECHNIQUES FOR  
THE LABORATORY DIAGNOSIS OF POLIO/ENTEROVIRUSES AND  
MEASLES/RUBELLA VIRUSES IN THE WESTERN PACIFIC**

19-23 May 2014, Hong Kong Special Administrative Region

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## **NOTE**

The views expressed in this report are those of the participants of the Hands-on Training Workshop on Cell Culture Techniques for the Laboratory Diagnosis of Polio/Enteroviruses and Measles/Rubella in the Western Pacific Region and do not necessarily reflect the policies of the World Health Organization.

This report has been printed by the World Health Organization Regional Office for the Western Pacific for the participants of the Hands-on Training Workshop on Cell Culture Techniques for the Laboratory Diagnosis of Polio/Enteroviruses and Measles/Rubella in the Western Pacific Region, which was held in Hong Kong, SAR, China from 19 to 23 May 2014.

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AND SECRETARIAT

Keywords:

Vaccines/ Poliovirus/ Enterovirus/ Cell culture techniques/ Measles virus/ Rubella virus/  
Health personnel-education/ Laboratories/

## SUMMARY

The Hands-on Training on Cell Culture Techniques for the Laboratory Diagnosis of Polio/Enteroviruses and Measles/Rubella Viruses in the Western Pacific Region was held at the Centre for Health Protection, Hong Kong Special Administrative Region (China), from 19 to 23 May 2014. Eighteen participants from eight countries took part in the workshop.

The objectives of the workshop were:

- 1) to enhance knowledge and skills of staff at national polio and measles/rubella laboratories on cell culture techniques of polio and measles/rubella viruses by:
  - i. maintaining cell lines for virus isolation based on WHO standard procedures;
  - ii. performing virus isolation and identification using the new algorithm for polioviruses and recommended standard procedures for measles and rubella viruses; and
  - iii. performing cell sensitivity for polioviruses on WHO recommended cell lines;
- 2) to further update the requirements for laboratory quality assurance (QA) of polio and measles/rubella as WHO network laboratories including proficiency testing, WHO accreditation, and data management and reporting.

The five-day training programme included lectures, practical sessions, country presentations, discussions and an evaluation of the workshop including an estimation of the participants' knowledge gain. The hands-on activities allowed participants to perform the cell culture and virus isolation techniques with facilitators, and thus, provided practical opportunities for participant to enhance their knowledge and skills.

## ABBREVIATIONS

AFP	acute flaccid paralysis
CPE	cytopathic effect
FTA	fast technology for analysis of nucleic acids
GLP	good laboratory practice
GSL	global specialized laboratory
hSLAM	human signaling lymphocytic activation molecule
ICA	Immunochromatographic assay
IgM	immunoglobulin M
IFA	immunofluorescence assay
IPV	inactivated polio vaccine
ITD	intratypic differentiation
L20B	mouse cell line (L-cells), genetically engineered to express the human poliovirus receptor
MeaNS	measles nucleotide surveillance
NIID	National Institute of Infectious Diseases
NSL	non-Sabin-like
NT	neutralization test
NPEV	non-polio enterovirus
OPV	oral polio vaccine
bOPV	bivalent oral polio vaccine
tOPV	trivalent oral polio vaccine
PHE	Public Health England
PHL	Public Health Laboratory
PHLC	Public Health Laboratory Centre
PT	proficiency test
PV	Poliovirus
QA	quality assurance
RD	Rhabdomyosarcoma
RNA	ribonucleic acid
RRL	regional reference laboratory
RT-PCR	reverse transcription–polymerase chain reaction
rRT-PCR	real time reverse transcription – polymerase chain reaction
RubeNS	rubella nucleotide surveillance
SIA	supplementary immunization activity
SOP	standard operating procedure
US CDC	United States Centers for Disease Control and Prevention
VDPV	vaccine-derived poliovirus
cVDPV	circulating vaccine-derived poliovirus
iVDPV	immunodeficient vaccine-derived poliovirus
VIDRL	Victorian Infectious Diseases Reference Laboratory
VPD	vaccine preventable disease
VTM	virus transport medium
WHO	World Health Organization
WPV	wild poliovirus

## 1. INTRODUCTION

The Western Pacific Region's polio and measles/rubella laboratory networks play a crucial role in maintaining polio-free status and in progress towards achieving regional goals of measles elimination by 2012 and accelerated rubella control by 2015.

To maintain polio-free status, polio network laboratories in the Region have been actively involved in acute flaccid paralysis (AFP) surveillance and supplementary enterovirus or environmental surveillance. Fast identification of polioviruses and vaccine-derived polioviruses (VDPVs) that can cause acute flaccid paralysis is becoming increasingly important because of reported circulating VDPV outbreaks in a small number of countries globally. In 2011–2012, 19 VDPVs were detected in China (n=17) and Viet Nam (n=2). With the introduction of the new algorithm for rapid detection of polioviruses, all 12 national polio laboratories (NPLs) in the Region and 31 subnational laboratories (SNLs) in China are using the new algorithm for virus isolation. Although all 43 polio network laboratories in the Region have successfully implemented the new algorithm, this workshop provided opportunities to share experiences and challenges in using the new algorithm and to improve the virus isolation techniques critical for the rapid detection and identification of polioviruses.

Laboratory performance will be critical in verifying national measles elimination. The recently published framework for verifying measles and rubella elimination<sup>1</sup> describes five lines of evidence to determine if a country or region has achieved measles and/or rubella elimination. Two of these lines relate directly to laboratory activities; the presence of high-quality epidemiologic and laboratory surveillance systems; and genotyping evidence that measles and rubella virus transmission is interrupted. It is recommended that laboratories submit sequence information for measles viruses to measles nucleotide sequence database (MeaNS) and for rubella viruses to rubella nucleotide sequence database (RubeNS) as soon as it is available.

As recommended at the Fourth Meeting on Vaccine-Preventable Diseases (VPD) Laboratory Networks in 2013, 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 standard molecular window and work in collaboration with the epidemiology groups to differentiate imported cases from endemic cases. Laboratories are encouraged to perform virus isolation and/or molecular detection of measles and rubella viruses including real-time reverse transcription polymerase chain reaction (RT-PCR) and sequencing to identify the genotypes.

### **1.1 Objectives**

- 1) To enhance knowledge and skills of staff at national polio and measles/rubella laboratories on cell culture techniques of polio and measles/rubella viruses by:
  - i. maintaining cell lines for virus isolation based on WHO standard procedures;
  - ii. performing virus isolation and identification using the new algorithm for polioviruses and recommended standard procedures for measles and rubella viruses; and
  - iii. performing cell sensitivity for polioviruses on WHO recommended cell lines;
- 2) To further update the requirements for laboratory quality assurance (QA) of polio and measles/rubella as WHO network laboratories including proficiency testing, WHO accreditation, and data management and reporting.

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<sup>1</sup> Framework for Verifying Elimination of Measles and Rubella, SAGE Working Group on Measles and Rubella (Draft of 18 October 2012)

## **1.2 Participants**

Eighteen participants from nine countries attended the training from WHO-designated national polio and measles/rubella laboratories in China (n=2), Malaysia (n=2), Mongolia (n=2), New Zealand (n=1), the Philippines (n=2), the Republic of Korea (n=2), Singapore (n=2), Viet Nam (Ha Noi and Ho Chi Minh City) (n=4), and Macao Special Administrative Region (SAR) China (n=1) as an observer. Facilitators were from the Polio Global Specialized Laboratory (GSL) in National Institute of Infectious Diseases (NIID), Tokyo, Japan; the Polio Regional Reference Laboratory (RRL) in Victorian Infectious Diseases Reference Laboratory (VIDRL), Melbourne, Australia; and WHO Regional Office for the Western Pacific. A full list of participants is available at Annex 1.

## **1.3 Programme**

The workshop included lectures, practical sessions, country presentations, discussions and pre- and post-evaluation. Due to commonalities in polio and measles/rubella laboratory based surveillance, concurrent lectures and practical sessions were conducted. Where there were differences in procedures, separate sessions were held for disease specialist participants.

The programme is available at Annex 1. The presentations are available on USB on request from WHO/WPRO EPI Data Team (wpr\_epidata@wpro.who.int).

## **2. PROCEEDINGS**

### **2.1 Opening session**

Dr Janice Lo opened the workshop and spoke of the importance of laboratory-based surveillance of polio and measles/rubella and the need to ensure good sensitivity for the detection of these viruses.

### **2.2 Overview of the global polio eradication and measles/rubella initiatives and regional status**

Dr Youngmee Jee emphasized that since WHO set the goal of polio eradication in 1988, great progress has been made. In 2014, wild poliovirus (WPV) transmission occurred in only three endemic countries (Afghanistan, Nigeria and Pakistan) and of the three poliovirus serotypes, only type 1 WPV is circulating. Type 2 WPV has been eradicated since 1999 and type 3 WPV has not been detected from 2013. From May 2013 to May 2014, key zones of active transmission occurred in Cameroon, Equatorial Guinea, Ethiopia, Iraq, Somalia and Syria. Thus, the WHO Regional Committee for the Eastern Mediterranean declared the new international spread of WPV an emergency for all Eastern Mediterranean Member States on 30 October 2013. The vaccination recommendations for travellers from polio-infected countries were updated.

In May 2012, the World Health Assembly declared the completion of poliovirus eradication to be a programmatic emergency for global public health and called for a comprehensive polio endgame strategy. In response, the *Polio Eradication and Endgame Strategic Plan 2013–2018* was developed. The plan recommends introduction of a single dose of inactivated polio vaccine (IPV) into the routine immunization schedule by the end of 2015 in all countries and a switch from trivalent oral polio vaccine (OPV) to bivalent OPV from 2016 to 2018. Among 194 Member States, 125 are using OPV, 50 are using IPV and 19 are using a sequential schedule. The Western Pacific Region has maintained its polio-free status since certification on 29 October 2000. All 43 polio network laboratories are functioning at a high level and have maintained full accreditation

status as of May 2014. WHO Regional Office for the Western Pacific publishes a biweekly Polio Bulletin to update polio laboratory network members.

The WHO Regional Committee for the Western Pacific established 2012 as the target year for measles elimination. The Expanded Program on Immunization (EPI), in the WHO Regional Office has developed two guidelines of practical strategies, innovative approaches in and lines of evidence in the progress towards elimination<sup>2</sup>. In March 2014, four countries were verified to have eliminated measles, namely: Australia, Macao SAR (China), Mongolia and the Republic of Korea. However in 2013–2014, resurgence of measles was reported from China, Papua New Guinea, the Philippines and Viet Nam. In 2013–2014, measles genotypes B3, D4, D8, D9, D11, G3 were detected while, Rubella genotypes 2B, 1E and 1J in 2013 and in 2014 (as of April), only 2B and 1E were detected. Despite challenges, the network laboratories continue to provide high-quality data to achieve the goal of measles elimination and rubella control in the Region.

## **2.3 Cell Culture**

### **2.3.1 Overview of Media for Cell culture**

Mr Kai Cheung Szeto presented the characteristics and composition of cell culture media to support the growth of cells and viruses. It is vital to pay attention to the characteristics necessary to the quality of cell culture assays such as: purity, authenticity, stability and sterility. All cell lines should be obtained from a reference laboratory or a reputable culture collection. Cell cultures serially passaged over an extended period of time will invariably show some signs of deterioration that will affect susceptibility of the cells to support the growth of the virus. It is strongly recommended that all cell lines used routinely (especially for polio) be replaced after a maximum of 15 sequential passages and constant supply of low passage cells from a master cell bank stored in liquid nitrogen should be available.

The choice of cell culture media is also essential, and significantly affects the success of cell culture. Cell culture medium generally is a mixture of components used to stimulate the natural environment of the cell that include inorganic salts, carbohydrates, vitamins, amino acids, lipids and trace elements, and serum. The sterility of the media and reagents should be ensured. It is recommended to use an autoclave to sterilize culture media that are heat-stable at 115°C for 10 minutes instead of 121°C for 20 minutes. While heat-labile reagents such as glutamine, glucose and trypsin should be filter-sterilized using 0.22 µm pore-size. Though media and reagents have been sterilized, cell culture can still be contaminated by bacteria, fungus, mycoplasma and viruses. One or more antibiotics can be used in conjunction with an antimycotic, however, viral contaminant is impossible to remove. Culture media also require specified temperature (4°C) for storage under specified conditions (away from light) and can be stored not longer than the shelf-life periods appropriate to each product. Proper labelling of culture medium and record keeping of cell passage are critical to maintain good documentation of cell culture. Cell lines require a temperature of 36°C for incubation and overheating (>39.5°C) can lead to cell death.

### **2.3.2 Review of Cell Culture Procedures (L20B and RD cells)**

Dr Hiroyuki Shimizu presented a review of the cell culture procedures for culturing enteroviruses. Poliovirus culture has standardized procedure on the use of two cell lines, L20B cells, a mouse cell line (L cells) genetically engineered to express the human poliovirus receptor that will support the growth of polioviruses but only a small number of other enteroviruses. The

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<sup>2</sup> Measles Elimination Field Guide (2013 Version)

<sup>3</sup> Guidelines on Verification of Measles Elimination in the Western Pacific Region (Draft), 2013

other WHO recommended cell line, RD cells were derived from a human rhabdomyosarcoma and are very sensitive to poliovirus but will also support the growth of many other enteroviruses.

The L20B cell line has a critical role to play in the polio laboratory network as its susceptibility to poliovirus is applied for the rapid identification of polioviruses, selective isolation of polioviruses and the accurate laboratory diagnosis of polioviruses.

Laboratories should rely on the supplemental *Polio Laboratory Manual* when dealing with all aspects of poliovirus culture. Polio laboratories should adapt any new L20B and RD cell lines received to local cell culture conditions and then establish a working cell bank (WCB). All cells recovered from the WCB should be used for virus culture for only 15 passages or three months, whichever is less. Cell sensitivity tests should be performed and critically, microbial and cross contamination (bacteria/fungi, mycoplasma) should be mitigated. Also, it is critical that contamination with other cells lines does not occur as this can lead to the reduction of virus sensitivity. The Polio LabNet has introduced annual virus isolation proficiency test that tests the laboratory's ability to both isolate and correctly identify polio and other enteroviruses.

### **2.3.3 Vero/hSLAM cells**

Mr David Featherstone presented a review of cell culture procedures for measles and rubella using Vero/hSLAM. Vero cells are susceptible to measles vaccine strains but not to wild-type measles virus. Dr Yanagi's group in Kyushu University developed a Vero cell line with a transfected gene for human signalling lymphocyte activation molecule or hSLAM receptor. Vero/hSLAM cell line has a very high sensitivity to both vaccine and wild-type measles. Rubella virus grows equally well in both Vero and Vero/hSLAM. However, for wild type virus and non-laboratory adapted viruses there is an absence of visible CPE. Vero/hSLAM is the recommended standard cell line for virus isolation in the Measles and Rubella LabNet because it supports the growth of both measles and rubella virus. The hSLAM receptor transfection in Vero/hSLAM has a Geneticin resistance gene linked to it. Geneticin is toxic to all cells without the resistance gene (per se, the hSLAM gene), so, any Vero/hSLAM cell which loses its receptor (and sensitivity to being infected with wild type measles virus) will be killed in the presence of Geneticin. However, transfection of hSLAM is very stable and there is evidence that Vero/hSLAM cells maintain the receptor for more than 50 passages in the absence of Geneticin. As Geneticin is an expensive antibiotic, the Measles and Rubella LabNet recommends that Vero/hSLAM be passaged with Geneticin for the preparation of stocks for storage in Liquid nitrogen. After resuscitation from liquid nitrogen, passaging in presence of Geneticin is not necessary. Vero/hSLAM cells should be passaged for only 15 passages after recovery from liquid nitrogen to ensure maximum sensitivity is maintained.

Unlike the Polio LabNet, a standardized susceptibility testing of Vero/hSLAM cells is not recommended by WHO as measles and rubella virus isolation is not a diagnostic tool and the large number of laboratories make the sending of stock virus cultures to all laboratories a logistical challenge and potentially very expensive. However, it is possible to monitor crude sensitivity using the growth of a low passage, wild-type measles virus of known sequence, which can be titrated and frozen in aliquots. Evidence of cytopathic effect (CPE) will determine presence of hSLAM receptor and comparing titres with the original virus stocks can be used for determining relative sensitivity. If virus sensitivity is suspected or proven, then, possible reasons to be addressed include: ensuring that correct media components are used, incubation temperature is optimally 37°C for measles virus and 35°C for rubella virus, regular check for mycoplasma contamination, ensuring cells are derived from WHO recommended sources and that if replacement cells are required, request them from GSL or RRL (only after all other options have been addressed).

#### **2.3.4 Algorithm for specimen processing and virus isolation including stool specimen processing and inoculation (Polio)**

Dr Hiroyuki Shimizu reviewed the algorithm for stool specimen processing, virus isolation, classification and physical characteristics of polio and other enteroviruses and the importance of maintaining viability of the virus from patient to the laboratory. An adequate volume should be collected: approximately 8 gm (2 thumbnails size), 2 samples, 24–48 hours apart, less than 14 days after onset, use of leak-proof container with a screw cap. For the data, samples should be labelled with patient ID and date of collection and sealed in a leak-proof bag. The completed patient history form should be sealed in a separate bag and the sample should be stored at <8°C until ready to be sent, or frozen if longer than a few days. The samples should be sent to the nominated laboratory under reverse cold-chain by the best transport mechanism determined. The laboratory should be notified of sample transport details to ensure follow-up if the shipment is delayed.

The new algorithm for polio detection reduces the cell culture steps from 28 to 14 days. There are changes in how CPE positive cultures are handled and omission of routine serotyping by neutralization test (NT) before ITD. A passage of all L20B isolates is required into RD before ITD to boost virus titres. The ITD step is reduced from 14 to 7 days through simultaneous serotyping and ITD by real-time RT-PCR. An immediate reporting of any poliovirus (PV) with non-Sabin-like (NSL) reaction is required and referral for VP1 sequencing. A separation of PV mixtures by neutralization test is required for sequencing. A diagram describing the process is found in Figure S1.1 supplement to the WHO *Polio Laboratory Manual*.

#### **2.3.5 Algorithm for specimen processing and virus isolation including specimen processing (Throat swab/urine specimens) and inoculation (Measles/Rubella)**

Mr David Featherstone presented the algorithm for specimen processing and virus isolation for measles and rubella. IgM detection in a single sample collected at first contact with a health facility is the standard procedure for confirming suspected measles and rubella cases. It is rarely useful to diagnose measles or rubella by viral culture or molecular detection. However, virus culture or molecular detection is important for molecular epidemiologic surveillance of measles and rubella viruses.

For the collection of clinical specimens for measles and rubella virus isolation, the most convenient and sensitive are: throat or nasal swabs for measles and rubella, and urine samples for measles only. Also suitable, but more difficult to collect, are nasopharyngeal aspirates and lymphocytes separated from heparinized whole blood samples. To maintain viability and enhance sensitivity for detection, specimens for virus isolation should be obtained as soon as possible after the onset of rash and within 5 days of onset and maintained under cold chain at all times from collection to arrival in the laboratory. Samples for virus isolation should be collected in addition to a serum sample but should never be substituted for serum samples. Measles and rubella viruses are highly temperature labile and susceptible to desiccation. Also, measles virus is closely cell associated. Throat or oropharyngeal swabs should be immediately added to viral transport medium (VTM) to prevent desiccation and maintain viability. Urine samples can be sent as they are without any processing as long as the storage and shipment is 4-8°C (not frozen) and within 3 days of collection. Longer-term storage should only be after centrifugation and re-suspension of the cell containing virus sediment in VTM or culture media. Long-term storage temperature should be at -70°C or lower, and never at -20°C.

Inoculation of Vero/hSLAM for isolation of measles and rubella virus requires the use of 25cm<sup>2</sup> flask with cells at approximately 85-90% confluency and at least one day after seeding. Overgrown cells are not receptive to virus replication. The preferred media for virus growth is Dulbecco's Modified Eagle Medium (DMEM) with 2% fetal bovine serum (FBS). Inoculated cells should be checked for CPE daily and all evidence documented. Any infected Vero/hSLAM cells should be passaged by trypsinization after 4-5 days, at a 1:3 split ratio (2nd passage). If no CPE is

detected after 4-5 days perform a “blind” 2nd passage at a split ratio of 1:3. Check the flasks daily. If no CPE is observed for 4-5 days after 2nd passage then perform rubella IFA, ICA or RT-PCR. If CPE is visible, continue to incubate until the CPE becomes extensive. It may be necessary to passage the cells one more time to allow the infection to spread. When CPE is visible over at least 50-75% of the cell layer, cells can be harvested for preparation of a viral stock. For controls, always include a negative control of uninfected Vero/hSLAM during virus isolation attempts. It is not recommended to use a positive control unless extreme caution is taken, as the risk of cross contamination is very high. If a positive control is used, it should be of a wild-type virus with a known genetic characterization.

For rubella virus culture, the same procedure for measles virus can be followed, however, 35°C is preferred as some rubella strains grow best at this temperature. A temperature of 37°C is acceptable if 35°C incubator is not available. Unlike measles, rubella virions are released into the media. Therefore, media or cells from infected cultures can be used to transfer the virus to the next passage.

### **2.3.6 Documentation for cell culture and virus isolation (Polio)**

Dr Bruce Thorley shared the documentation for cell culture procedures for poliovirus. Worksheets are essential to allow the recording of results in a systematic format. Data collection should be consistent, accurate and concise to enable work procedures to be traced. A controlled documentation trail means laboratory records clearly show; what, how and by whom the test was done, what equipment and/or reagents were used, what results were obtained and the follow-up, who reported the result and if problems were encountered, how they were addressed. There also needs to be an established and documented trail of data from receipt of a specimen in the laboratory to reporting of the final result.

### **2.3.7 Documentation/Worksheets for cell culture and virus isolation (Measles/Rubella)**

Mr David Featherstone described the similarity in principles for documenting polio and measles/rubella processes. Participants reviewed an excel sheet including functions for monitoring different processes.

## **2.4 Cell sensitivity and authentication of cell lines**

### **2.4.1 Evaluation of cell sensitivity and authentication of cell lines**

Dr Shimizu made a presentation by Dr Bruce Thorley on Cell Sensitivity and Authentication of Cell Lines. Contamination of cell lines by other cell lines can be difficult to detect but can have a profound impact on the sensitivity of the standard cell lines. Contamination may manifest itself through high rates of NPEVs in L20B cells, for example, growth of echovirus 9 in L20B.

To detect cell contamination, DNA barcoding can be used to identify cell line species based on 5' end of cytochrome oxidase subunit 1 mitochondrial gene. This is a 648 bp region flanked by conserved regions which allows for the design of PCR primers, and variable sequence differentiates species. Another method is to use the phylogenetic analysis of cells and use RT-PCR with species specific primers to allow sensitive detection of 1 RD cell in 10<sup>6</sup> L20B cells.

For virus sensitivity, National Institute of Biological Standards and Control (NIBSC) has distributed Sabin reference strains to all polio laboratories from standard stocks. These Sabin reference strains are used by laboratories to develop Laboratory Quality Control (LQC) standards of virus at a known titre in relation to the NIBSC stocks. Laboratories are required to test the sensitivity of L20B and RD-A cell lines for poliovirus infection on a regular basis. The National Polio Laboratory determines the titre of aliquots of NIBSC authenticated Sabin strain and if titre is +/- 0.5 logs of titres quoted in WHO laboratory manual, the test is considered valid and the

outcome is that the cell line is suitable for routine use. If the titre for the authenticated Sabin is not within +/- 0.5 log of the NIBSC titre, the laboratory should repeat the cell sensitivity test, adjusting the dilution range. If it still does not meet the minimum requirements, then, new cell lines should be requested from the designated RRL cell bank.

A working cell bank (WCB) should be established from cells where the flask is 80-100% confluency to maintain the cells in an exponential log phase. Cells should not be permitted to become over-confluent as they go into lag phase. The WCB should be kept to a low passage number, about two to three passages after receipt from designated cell repository. A test cryovial of the cells should be revived and cell sensitivity performed. Cell sensitivity should be carried out at about the mid-point (7 or 8) in the cell-line's 15 passage recommended lifespan. Cell sensitivity testing performed at the end of the 15 passages is also optional.

#### **2.4.2 Worksheets and submission of cell sensitivity reports to WHO**

Dr Youngmee Jee presented the monitoring of cell sensitivity testing for the laboratories in the Western Pacific Region Polio LabNet. The 4th VPD meeting recommendations from 2013 were: 1) all network laboratories should conduct cell sensitivity testing midway through the expected use of 15 passages (at least every 3 months) and report results to the WHO Regional Office for the Western Pacific within 48 hours of test completion for review and for implementation of appropriate corrective actions; and 2) the results should be presented in a graphic format by serotype for easy monitoring of trends.

The regional results show that 11/11 (non-China) laboratories are reporting from 2013 and 30/31 China provincial laboratory results regularly reported to WHO Regional Office by China CDC. Performance issues identified from analysis of the data include: more than 15 or unlimited passage for L20B and RD cells; decreasing linear trend of CPE ratio not observed: dilution problem; low titres of laboratory quality standard (LQC); invalid titration results (not showing 100% and 0% CPE for end point dilutions) without corrective action; stock-out of low passage L20B and RD cells; continuous use of both NIBSC and LQC strains at the same time; use of cells beyond recommended passage limit (L20B>p35, RD>p245); not performed at midway (p.7-8), used cells of any available passages; irregular cell sensitivity testing due to high workload for other disease surveillance and staff absence; and delay in sharing results with WHO.

#### **2.5 Shipment of specimens/isolates to Regional Reference Laboratory**

##### **2.5.1 Standard operating procedure, packing and shipment requirements**

Dr Stanley Chan presented requirements for the safe packing and shipment of specimens and virus isolates. Laboratories should ensure the safe transportation of specimens and isolates to prevent unnecessary delays, loss of viability of specimens, or increase the risk of accidental exposure to infectious materials. Planning is important and advance arrangements should be made with the receiver to determine whether any import permit is needed, a mutually convenient time for shipment. Advance arrangement should also be made with the courier to ensure the shipment is accepted, sent by the most direct routing, well documented, shipment conditions monitored and any delays notified.

Under the International Air Transport Association (IATA) regulations to classify materials, poliovirus is classified as category A in Division 6.2 for infectious substances and measles virus as category B. Category A infectious substances are capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals if exposure occurs during transport. This is classified as UN2814 (Infectious substance affecting humans). Diagnostic samples are considered exempt if they are human (or animal) specimens and there is minimal likelihood that pathogens are present. These are not subject to dangerous goods requirements and regulations if they are transported in packaging that prevents leakage and are correctly labelled.

For follow-up after shipping, the laboratory must: notify the receiver when the shipment has been sent, request the receiver to notify the sender when the shipment arrives and to report any problems encountered.

### **2.5.2 Use of FTA cards: Procedure for spotting and storage and extraction**

A CDC video on the extraction of nucleic acids from Whatman FTA (fast technology for analysis of nucleic acids) cards was shown to all participants after the practical demonstration of preparing samples of virus nucleic acid for safe and stabilized shipment to the RRL or GSL for molecular testing.

## **2.6 Good Laboratory Practices and Quality Assurance**

### **2.6.1 Cell culture/Virus Isolation/Molecular Laboratories**

Dr. Janice Lo described Good Laboratory Practice (GLP) as promoting the quality and validity of test data. GLP is also a managerial concept of the organizational process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. The elements of GLP and the requirement for QA and ISO 15189 management and technical requirements were described. QA processes should be implemented to ensure sensitive and accurate results. Control for false positive and false negative results is also important.

### **2.6.2 Standard Operating Procedures**

Dr Bruce Thorley presented Standard Operating Procedures (SOPs) and Quality Management Systems. An SOP should describe the purpose of the test, it should be clear, unambiguous and able to be understood by staff even without experience. The SOP should also describe specific biosafety hazards and precautions that need to be taken (Material Safety Data Sheet [MSDS]), define any worksheet to record data if appropriate and all references used to develop it should be listed. Any procedures described should be fully consistent with WHO laboratory manuals. All SOPs should be approved by laboratory management, reviewed regularly and updated if necessary. The layout of the document should include a header with identifier code, version number, date of original SOP and any update.

GLP generates consistent and accurate results and these define the quality of an organization. A Quality System Manual defines organizational structure, provides a framework for meeting quality system requirements and conveys managerial commitment and states what an organization does and how it puts it into practice. WHO, CDC and other handbooks on Laboratory Quality Management Systems (LQMS) were discussed, along with LQMS components and how these relate to the polio and measles labnets, including using regular proficiency testing and accreditation programmes to measure performance of laboratories.

### **2.6.3 Equipment maintenance and inventory**

Mr David Featherstone discussed laboratory equipment – much of which is vital for laboratory operations. Keeping equipment operating well is critical. Preventive maintenance can save money and time – rather than waiting until equipment stops performing properly. Preventative and regular maintenance schedules should be planned and documented for each piece of equipment. The process should start with the selection and purchase of the equipment. Prior to purchase, questions to ask include:

- What level of maintenance is required to keep it functioning?
- Are spare parts readily available?
- Is a manual available?
- Are special tools or instruments required to perform in-house maintenance?
- Is there a local service agent?

- Have other laboratories used the same or similar equipment? What was their experience? (as a guide for whether the equipment will perform well)

Price should not be the only determining factor for purchase as cheaper equipment may be sub-standard or may not include a service agreement. It may be possible to build a maintenance contract into the purchase price, which will make the initial cost more expensive but may generate greater savings over the long term. Other considerations in the purchase process include: ensuring all validation and safety equipment needed for equipment is included, uninterruptible power supply (UPS) or voltage regulator with all connectors are provided if appropriate, and that installation by specialists may be required.

For the use and maintenance of equipment, it is critical to document all steps. An SOP describing the equipment, its use and the maintenance required should be developed along with an inventory of all equipment used in the laboratory with appropriate maintenance records, validation processes for equipment and of the monitoring devices used, and a log of the use of equipment. WHO has developed a series of documents to help with these processes that were included in the list of references on the USB provided to all participants.

#### **2.6.4 Review of the Polio Laboratory Accreditation Checklist (Performance Indicators), External Quality Assurance: Proficiency Test**

Dr Bruce Thorley presented the polio isolation proficiency testing programme with a focus on the 2012 and 2013 PT results and the plans for the 2014 PT. The polio isolation PT was prepared by the GSL at Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Netherlands. The samples are simulated stools stabilized with magnesium chloride (MgCl<sub>2</sub>) and shipped at ambient temperature. All shipments were reportedly received in good condition. Of the shipment for polio laboratories in the Western Pacific Region, 8 of 10 shipments were received within 2 days. The other two were received after 3 days. The basic principles of scoring the PT were described.

Only NPEVs growing on RD are scored as NPEV. NPEVs growing on other cell lines only are scored as “other enteric viruses”. Scoring of results for poliovirus isolation PT was according to the concepts of the new algorithm and a passing score was defined as 90%.

The Western Pacific Region laboratory results for 2012 were summarized as: all laboratories reported results within 14 days, all laboratories reported correct results except one laboratory which observed L20B positive (1+) with NPEV and scored 90%. Coxsackie virus B1 (CB1) was not isolated in RD by one laboratory, but, no marks deducted due to inconsistent growth in this cell line. No extra marks are awarded for additional testing – there is a debate whether it confers and unfair advantage compared to routine laboratory procedures. It was recommended that laboratories should base their PT results on cell culture observations as carried out in their routine testing.

The 2013 isolation PT was received at VIDRL in November 2013. However, distribution to Western Pacific Region laboratories was delayed by VIDRL's relocation to the Doherty Institute. The PT was distributed to six laboratories in May 2014 and results reported by two of them. Two shipments are in transit (Japan and Singapore) and two shipments are booked for the week following the workshop (Republic of Korea and Mongolia).

For the 2014 PT, the date of dispatch from RIVM is indefinite, so, there can be no prediction of when VIDRL can ship to the laboratories in the Western Pacific Region. VIDRL has received four WHO proficiency panels six weeks before the end of the year: polio isolation, ITD RT-PCR, VDPV RT-PCR and sequencing, which makes it challenging to ship to all laboratories by the end of the year. Dr Thorley recommended that GSL coordinate the timing of proficiency panel shipments, so that they do not all arrive at the same time of year.

### **2.6.5 Review of Measles/Rubella Accreditation Checklist (Performance Indicators), External Quality Assurance: Proficiency Test**

David Featherstone presented to the measles laboratory participants on the recent changes in the accreditation checklist and procedures. All participants were familiar with the checklist, so, the focus of the presentation was the changes recommended at the Global Measles/Rubella LabNet meeting in 2013. Reasons for the changes include: global standards for monitoring progress towards and achievement of elimination have essential LabNet components which need to be reflected in the review checklist, the introduction of the new molecular PT programme, strengthening of QA/QC activities, strengthening the need for SOPs, use of approved and validated kits are monitored, clarification of some language and as part of the regular periodic review process.

As laboratory surveillance is an integral part of disease surveillance, full awareness of the performance of the rest of the surveillance programme and knowledge of progress towards measles and rubella elimination goals is important. As such, a summary of country-specific surveillance summary data is examined under a new Part IV, including vaccine coverage, rates of suspected measles detected, reporting rates, and cases reported in the categories of clinically confirmed, laboratory confirmed and epidemiologically linked, with an incidence of all confirmed cases.

The frequency and process of accreditation reviews will change in some countries. All laboratories will be required to fill out the accreditation checklist part I to IV in the first quarter of every year, for the preceding calendar year. Regions will collate and provide a summary of performance indicators to WHO headquarters for all regional laboratories by the end of the first quarter. WHO headquarters will provide a summary of global laboratory accreditation results by the end of April for the WHO annual report and LabNet SharePoint. The onsite review component of the accreditation assessment carried out by WHO will be periodic, from every 1–4 years depending on the results of the paper-based review.

### **2.6.6 Intratypic differentiation of poliovirus: wild/vaccine/VDPV and reporting**

Dr Hiroyuki Shimizu presented the ITD of polioviruses and their reporting. The ITD real-time RT-PCR protocol used in the Polio LabNet provides the following evidence of possible causes of CPE in L20B/RD cells:

- Viruses or NOT
- PEV (including poliovirus) or NOT
- Poliovirus or NOT
- Poliovirus serotype (type 1, 2 or 3)
- Single serotype or mixture
- Vaccine-like (Sabin-like) or NOT
- Vaccine-derived poliovirus (VDPV) or SL (differentiated by the VDPV real-time PCR)

VP1 sequencing is required to determine WPV (or not) and the genetic lineage of WPVs and VDPVs. The VDPV definition arose from recommendations of the 16th Informal Consultation of the Global Polio Laboratory Network in 2010. The definition of circulating vaccine-derived poliovirus (cVDPV) is VDPV associated with two or more cases of AFP. VDPVs for type 1 and type 3 require greater than or equal to 10/906 nucleotides (nt) in VP1, different from Sabin 1 or Sabin 3 respectively. VDPVs for type 2 poliovirus are defined as those viruses with greater than or equal to 6/903 nt different from Sabin 2. New real-time ITD and VDPV screening algorithm has been introduced in the Region. The new algorithm was recommended for adoption in the Polio LabNet in 2009 with two real-time RT-PCR procedures implemented. VIDRL hosted training for the Region in 2009.

## **2.7 Molecular genotyping of measles and rubella isolates and Reporting**

Mr David Featherstone presented measles and rubella genotyping and reporting. Molecular surveillance is a powerful tool in the measles and rubella surveillance programme as sequence information, in combination with epidemiological data can: allow mapping of transmission pathways, identify possible source of virus and determine whether it is indigenous or from an imported source, determine whether suspected case due to vaccine or wild virus, assist with confirmation of true positives and improve diagnostic resolution in first few days after rash onset in combination with IgM (especially for rubella).

Identifying the geographical origin and tracing the transmission pathways of a virus allows the programme to build a comprehensive knowledge of global distribution of the spread of virus strains and the source of virus in new outbreaks may be determined as imported or indigenous. However, sequence information alone may not be sufficient to identify the source of an infection or transmission pathways and epidemiological investigations, especially in combination with molecular information can identify the likely source of origin of virus and/or transmission pathways.

For the measles virus, N and H genes are the most variable regions on the genome. The standard region for sequencing is the 450nt coding for the COOH terminal 150 amino acids of the N protein from all specimens and is the minimum sequence needed for reporting genotype information. The entire H gene is the secondary region for sequencing of selected measles virus isolates and provides for greater resolution in molecular analysis. WHO reference strains from each of the 24 known genotypes are available to make a genotype assignment. The list of WHO reference strains is found in the WHO, Weekly Epidemiological Record (WER) for 2005.

For rubella virus, the recommended minimum sequencing window is 739 nts (between 8731 and 8469 nts) of the more variable region of the E1 structural gene. As for measles virus, rubella reference strains are available for the assignment into one of the 13 genotypes.

Laboratories play a critical role in determining progress towards elimination by providing evidence of transmission patterns, and helping to define when measles is eliminated and when the virus is reintroduced.

WHO and PHE have developed two sequence databases, MeaNS for measles viruses developed in 2008 and RubeNS for rubella virus developed in 2012. More than 16 000 measles N450 sequences have been submitted to MeaNS and 1041 rubella virus sequences have been submitted to RubeNS. Laboratories were strongly recommended to submit their sequence data to the two databases as they also provide many tools to assist with naming the virus, providing exact matching, BLAST matching, genotyping and phylogeny tool and easy submission to GenBank. In MeaNS, submitters can also develop maps and charts summarizing global trends of genotypes of viruses submitted.

## **2.8 Biorisk Management**

Dr Bruce Thorley discussed the need for biorisk management in LabNet. Training in Biorisk Management aims to promote biosafety and biosecurity awareness in the global laboratory networks including recent developments, aids professional development, improves practices and can instigate change to management practices. Laboratory biosafety is the containment principles, technologies, and practices implemented to prevent unintentional exposure to pathogens and toxins, or their unintentional release. Laboratory biosecurity refers to protection, control and accountability for valuable biological materials within laboratories to prevent their unauthorized access, loss, theft, misuse, diversion, or intentional release.

Laboratory biorisk management is a system or process to control safety and security risks associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities. The risk associated with biological materials in the laboratory has a safety and a security component and biorisk encompasses risks from the biosafety and laboratory biosecurity perspective, associated with biological materials. There are three components for the identification and management of biological risks: How they are identified (Assessment), the steps taken to manage these risks (Mitigation) and determining how the risk management is working, and will continue to work (Performance).

A Biosafety risk assessment model is available at: <http://www.biosecurity.sandia.gov/BioRAM/>. The model is available for institutional biorisk assessments which have questions and details for making laboratory safety/security decisions. There are four categories of mitigation control measures: Engineering Controls, Administrative Controls, Practices and Procedures and Personal Protective Equipment. In implementing mitigation controls the laboratory should first consider elimination or substitution. A combination of control measures should be used based on their effectiveness and laboratory ability to implement them. These should be based on the results of the risk assessment, and should provide maximum impact.

## **2.9 Country reports**

### **2.9.1 Measles and Rubella Network Laboratories**

#### *China*

Dr Mao Naiying presented the status of the National Measles Laboratory at China CDC, and an update on the progress towards achieving measles elimination in China. In 1965, a domestically produced liquid measles vaccine was introduced in China. Prior to this, incidence of measles in China was about 590/100 000. In 1986, a freeze-dried measles vaccine and a two-dose immunization schedule were introduced. In 2001, the measles laboratory network (MLN) was established which comprised the national measles laboratory (NML, WHO regional reference laboratory), 31 provincial laboratories and 331 prefecture laboratories. In 2004, the schedule of second dose of measles vaccine was changed from 7 years old to 18 months. In 2010, a nationwide measles immunization campaign was launched with more than 100 million children vaccinated. In 2013, a molecular method (real-time RT-PCT) was introduced for measles and rubella detection in provincial and prefecture laboratories.

China achieved good control of measles after the nation-wide SIA in 2010, but in 2014, a resurgence occurred with 13 217 measles cases reported in 2014 up to March – an increase of 76.2% compared to the same period in 2013. The age distribution of measles cases from January to March 2014 was predominantly in the under 1 year and 15–50 years age groups.

Virological surveillance in China showed that from 1993 to 2013, 5163 measles viruses were isolated by China Measles/Rubella Laboratory Network and 5013 of them were identified as H1a genotype except for 65 D9, 46 D8, 31 Vaccine, 3 D11, 3 B3, 1 H2 and 1 D4. In 2013, 2322 measles strains were isolated from 29 provinces, 2208 isolates identified as H1a genotype, 13 isolates identified as VAC, 51 as D8, 47 as D9 and 3 as B3. Up to March 2014, 559 measles viruses were isolated and 555 were identified as H1a except for 2 D9, 1 G3 and 1 Vaccine with measles virus sequence information was obtained from all provinces except Tibet.

Measles H1 genotype is still the predominant endemic genotype in 2014 in China with imported D9 and G3 genotypes found in Yunnan and Guangdong provinces, respectively.

For rubella virus sequence information, from 1999 to 2013, 1023 rubella isolates were obtained from 18 provinces with 4 genotypes identified: 1E, 1F, 2A, 2B (Table 3). In 2013, 177 Rubella virus isolates from 16 provinces were detected.

Proficiency testing in the China LabNet: In 2013, the Global measles and rubella serological proficiency test panel consisting of 20 coded sera were tested by 30/31 provincial Measles/Rubella Laboratories of China (Tibet did not participate). Two commercial kits were the most commonly used for measles IgM testing, Haitai and Virion/Serion. Two provinces (Hebei and Xinjiang) recorded results with significant lower sensitivity from other provincial laboratories. However qualitatively, all provinces achieved 100% score. For rubella, three major kits were used; Haitai, Kerunda, and Virion/Serion and all participating provinces achieved 100% score.

For confirmatory testing, 930 serum samples were sent to NML for confirmatory test in 2013 by 30/31 provinces (exception Tibet). Each provincial measles laboratory sends 30 sera (10 measles IgM positive sera; 10 rubella IgM positive sera and 10 negative sera) to NML for confirmatory testing. Two measles and three rubella commercial IgM ELISA kits were used as recommended by 2012 onsite review for the confirmatory test. All except two provinces achieved 100% scores.

Challenges affecting the China LabNet include absences of gene data from some regions in China and these were: inconsistent results between serologic test and real-time RT-PCR for measles diagnosis was found in some province laboratory; documentation of training and trouble shooting for network laboratories conducting real-time RT-PCR test; quality control for real-time RT-PCR for provincial and prefecture laboratories.

#### *Hong Kong SAR (China)*

Dr. Janice Lo presented the status of measles and rubella – outlining milestones for Hong Kong SAR (China)'s implementation of measles and rubella control:

- 1967: Measles vaccine introduced (one dose)
- 1978: Rubella vaccine introduced for primary 6 girls
- 1990: MMR vaccine introduced (one dose)
- 1996: MMR vaccine introduced (2<sup>nd</sup> dose at primary 6)
- 1997: MMR vaccine introduced (2<sup>nd</sup> dose at primary 1)
- 1997: Special campaign for 1-19 years of age with
- MMR coverage currently reported as over 95%

For surveillance of measles and rubella, in 1961, measles was made notifiable and in 1978 CRS became voluntarily reportable. In 1994, rubella was made notifiable and in 2008 CRS became notifiable. Timeliness of reporting was >90% for every year from 2009-2014.

Measles sequencing results from 2009 to 2014 showed four genotypes ( B3, D8, D9 and H1) detected and all were considered due to importation and many had epidemiological links to other countries. Rubella genotypes detected from 2009-2014 were either 1E or 2B with the exception of N=2 1J viruses detected in 2010.

A measles serosurvey was carried out by the NML in 2010 showing >96% positivity using Human, Siemens IgG and PRNT neutralising assays.

The RRL provides a critical role for the LabNet in confirmatory testing of samples from the eight NMLs under its responsibilities. It also carries out direct sequencing on select serum samples and been able to provide evidence of measles strains in countries which have no other genotype information. Other RRL responsibilities the NML performs are: retesting of serum specimens for concordance of IgM results, confirmation of culture results, PCR and genotyping and phylogenetic analysis.

Challenges include maintaining high MMR vaccination coverage; vigilance of frontline clinical staff for infection control; and maintaining timeliness in laboratory reporting (both as an NRL and as an RRL).

#### *Malaysia*

Ms W Nur Afiza bt. W. Mohd Arifin presented the measles elimination programme in Malaysia. In 2011, four deaths in State of Sabah involving foreigners were reported. No measles death was reported in 2012 and 2013.

Measles incidence has decreased since the introduction of MMR. However, there was a notable increase in measles cases due to outbreaks in 2011 (n=4910) and 2012 (n=8535). In 2011, 1445 cases (29.43%) were laboratory confirmed. In 2012, 1,655 cases (19.4%) were laboratory confirmed. Though 6958 measles cases were reported in 2013, only 175 cases (2.5%) were laboratory confirmed. The majority of the measles cases were children under-7 years and were not vaccinated.

The laboratory has implemented laboratory QA and maintained high-quality performance. The laboratory performs virus isolation and real-time PCR for virus detection and also genotyping. Measles genotyping data showed D8 and D9 continue to circulate from 2011–2013 and rubella genotypes detected from 2011–2013 were 2B and 1E.

Malaysia has appointed Kota Kinabalu Public Health Laboratory as a measles subnational laboratory in Sabah to conduct serology testing for measles and rubella IgM for East Malaysia. NPHL is monitoring PHL's quality of testing to ensure adherence to WHO requirements. The PHL started measles and rubella IgM testing in June 2011. The PHL is also implementing QA programmes and performs well based on WHO standards.

Malaysia has reduced the number of underreported cases. SIAs have shown positive results as a decreasing trend of cases are seen since the activities were carried out extensively. Challenges identified were: the need to send serology samples for all suspected measles cases must be fulfilled as it is a requirement under the enhanced case surveillance. However the number of notified cases and sample send for laboratory testing are markedly different; obtaining a second sample for equivocal cases is not always successful; and collection of specimen for virus isolation must be strengthened to ensure identification of the measles virus genotype in each cluster.

#### *Mongolia*

Dr Nyamaa Gunregjav presented the history of measles elimination in Mongolia. From 1964 to 1992, 6320 cases were reported in Mongolia with 79 morbidity cases. The one dose schedule of measles containing vaccine (MCV) was introduced in 1973, and the two dose schedule of MCV was introduced in 1986 national immunization days (NID) in 1994, 1996 and 2007, and subnational immunization days in 2000 were conducted and the coverage was very high (>90%). In 1998, a mandatory reporting system of measles started. Genotype data have been available since 2001. MMR vaccination was introduced in 2009 replacing monovalent MCV and is provided for children aged nine months and two years old.

The laboratory has been accredited by WHO since 2004 as the national measles laboratory. Measles case-based surveillance started in 2008. From 2010 to 2013, no measles case was reported. In March 2014, the Regional Verification Commission has verified that Mongolia has interrupted endemic measles virus transmission for at least 36 months from June 2010.

The laboratory has the capacity to perform serology, virus culture using Vero/hSLAM cells and molecular detection by PCR methods including genotyping. Rubella genotype during 2010–2013 was 1E. The laboratory conducted mumps diagnosis, and the mumps IgM positive rate was very high at 55% in 2013. Mumps genotype strain H3 was detected in 2009 and 2011, while

mumps genotype F was detected in 2011–2012. The laboratory participated in various WHO QA programmes and scored 100% in recent confirmatory and proficiency testing. Molecular epidemiologic surveillance of measles and rubella is an ongoing challenge.

#### *New Zealand*

Ms Jacqueline Ralston presented the updates of measles activities in New Zealand. Between December 2013 and 24 April 2014, there have been 125 measles cases have been reported in New Zealand. Of these, 104 were in Auckland, 15 in Bay of Plenty/Lakes, four in Wellington and two in Hawke's Bay. Twelve of these cases acquired measles while travelling overseas and the rest are mostly contacts of these cases. During 2009, measles outbreaks occurred in Christchurch (D4 strain). In 2011, three importations of measles virus (D9, D8 and D4 strains) occurred from January to June. D9 importations into Auckland, Wellington and Christchurch were followed by D9 importation into Hawkes Bay and a D4 importation into the Auckland area. D4 importations in Auckland, Wellington (mini) and Hawkes Bay continued until July 2012. The measles genotype detected in 2014 was B3.

Measles Mumps Rubella (MMR) vaccine coverage for first dose is >90% on cohort of children from 2006–2011, while, MMR vaccine coverage for second dose is <90% for children born in 2006 and 2007. The cohorts of children born during 2008 are still completing their second dose in 2013 (scheduled for age 4).

New Zealand continues to have outbreaks of measles, although at lower rates than previously. Due to higher immunization rates in children by the age of 2 years, outbreaks particularly affect unvaccinated older children, teenagers and adults. The vaccines are free for children and adults who have not previously received 2 doses of measles-containing vaccine.

#### *Philippines*

Ms Herma Base from RITM presented the vaccination activities and measles/rubella cases from 1999 in the Philippines. Despite follow-up (door-to-door) measles immunization in 2004 and 2007, the Philippines had measles outbreak in 2010–2011 leading to the door-to-door measles–rubella SIAs targeting children 9 months to under-8 years from April to May 2011.

Following outbreaks in 2010 and 2011, a decreasing number of measles cases were reported in 2012. The Philippine government issued an Administrative Order to strengthen laboratory confirmation of all suspected measles cases in to improve measles surveillance. However, another outbreak occurred from May 2013 to the first quarter of 2014. The laboratory faced challenges including backlogs of samples for testing.

The Virology Department has activated its incident command system to provide human resources and maintain supplies/reagents. However, in February 2014 they noted extreme difficulty testing all samples. Priority testing of samples was implemented from: calamity areas (region 8 recently affected by Typhoon Haiyan), areas with no positive measles cases and areas that are considered “urgent/priority” by the regional units. Performance indicators in 2014 were below target. The laboratory results showed that majority of confirmed measles cases were found in children under-4 years.

The swab samples for virus isolation and PCR have also increased during the outbreak. Virus isolation (n=803) and real time PCR (n=872) of measles virus were done, showing 27.4% isolation rate and 92% detection rate by PCR. Also, 52 virus isolation negative samples were tested by PCR and 46 (88.5%) samples were measles positive. Samples for sequencing were sent to 1stBase Laboratory in Malaysia and analysis was done by the RITM molecular staff. The results showed that B3 measles genotype continues to circulate in the Philippines since its detection in 2013. The laboratory experienced measles kits stock-out in February 2014 and there was a mycoplasma contamination of Vero/hSLAM cell lines. Despite challenges faced, staff continue to find ways to improve their performance.

### *Republic of Korea*

Dr Haeji Kang presented the vaccination and surveillance activities in the Republic of Korea. WHO declared the elimination of measles in the Republic of Korea in April 2014. The high vaccination rate of MMR that exceeded 95% for more than 10 years has enabled the Republic of Korea to be measles-free. The recommended age for routine vaccination is 12–15 months for the first dose of MMR, and 4 to 6 years for the second dose of MMR.

The laboratory performs serology, virus isolation and molecular detection using real-time PCR and sequencing for confirming measles and rubella infection. Differential diagnoses for parvovirus B19 and human herpesvirus 6 (HHV-6) are also performed using Biotrin IgM EIA (parvovirus B19) and Panbio IgM EIA (HHV-6). From the outbreak in 2011, all RT-PCR measles positive cases were identified as D9 measles virus strain except one vaccine-related case. This was the first detection of D9 strains in the Republic of Korea, which was possibly related to importation from Philippines and Thailand. In 2012, no measles virus was detected from measles IgM positive sera tested by RT-PCR. Since it is difficult to collect good-quality serum samples and other samples such as throat swabs and urine. In 2013, measles genotype B3 (n=75) and D9 (n=2) were detected with 3 imported cases identified from Indonesia and Philippines. During 2014, as of April, B3 (n=85) still continue to circulate and new genotypes D8 (n=4) and H1 (n=1) were detected with 10 imported cases identified from China, the Philippines, Singapore and Viet Nam. Most of measles cases in the Republic of Korea in 2014 are less than 3 years old (49.3%) and unvaccinated or more than 20 years old (24.4%) with unknown vaccination history. Challenges include staff shortages, the need to train new staff for measles laboratory, the need to strengthen staff's activity in provincial laboratory for measles and rubella diagnosis, and maintaining the elimination of measles.

### *Singapore*

Dr Loh Pei Ling presented the measles situation in Singapore. Children receive two doses of the MMR vaccine. The first dose is given at 12 months and the second between 15 and 18 months. Measles cases must be reported to the Ministry of Health not later than 72 hours from the time of diagnosis. Since June 2012, enhanced our surveillance for measles and rubella cases was initiated by following up on each clinically diagnosed case. All clinically diagnosed cases that meet the clinical case definition will be offered testing. From January to December 2012, 37 measles cases were laboratory confirmed. In 2013, 139 cases were reported and 54 (39%) cases were laboratory confirmed. Among 141 cases reported between January–April 2014, 90 (63%) cases were laboratory confirmed and of these, 23 cases had a travel history to the Philippines which had measles outbreaks in January.

The NPHL is working with Ministry of Health on the measles surveillance workflow. Under the Singapore law (Private Hospitals and Medical Clinics Act), health-care institutions must establish one or more QA committees to monitor and evaluate the quality and appropriateness of the services provided and the practices and procedures carried out, hence, the laboratory ensures high-quality measles serology testing.

### *Viet Nam Hanoi*

Dr Phoung Loan DO from NIHE presented the measles situation in Viet Nam. Measles vaccine was introduced into the EPI programme from 1984. As a result of the high vaccination coverage, measles incidence decreased dramatically to less than 1 per 100 000 in 2012. However, outbreaks still occurred every two to three years but the peak was lower. Between 2013 and 12 May 2014, among more than 18 000 suspected cases, 4394 were confirmed. In 2013, outbreak occurred in some mountainous province having border with China and quickly spread to the whole country. In this outbreak, the highest risk age group was under 3 years, which were in the vaccinated age group. However, only 4.4% of these children received two doses of vaccine.

Children vaccinated with two doses was 86%. Vaccinated children with one dose was 95%. Among 129 deaths, 72.4% were under 1 year old, however, 19.4% of cases died due to measles.

In the laboratory, there are about 400 samples pending for testing and also focused in finding new cluster. In 2013, 143 swab specimens were tested for PCR and virus isolation. Of these, PCR results showed 59% measles, but the virus isolation rate was only 6.9%. However in 2014, of 138 swab specimens tested, the PCR positive rate was 94.4% and virus isolation rate was 41.9%. Measles genotype was H1. The Provincial Preventive Medicine Center (PMC) performed ELISA for measles and rubella. Many different ELISA kits are used (IBL, Serion, Biorad, Vircell, Siemens and others). As such, quality control in testing is of concern. The laboratory is planning to set-up the External Quality Assessment (EQA) programme for provincial laboratories. Reagents and ELISA kits are also running out.

#### *Viet Nam Ho Chi Minh City*

Dr Minh Hoang from Pasteur Institute, Ho Chi Minh City presented on measles and rubella surveillance in southern Viet Nam. From January to September 2013, no positive measles case was detected. However, measles outbreak occurred during October 2013–April 2014, with the highest peak in February (n=559 cases) and March (n=1006 cases).

Most of the measles cases were males (53.6%) and 42.4% were female. Among the measles cases, 41.4% were children under 1 year old, 29.3% were children aged 1 year and under-6 years old, and 24.3% were children over 6 years old. No rubella positive case was detected from January to July 2013, though, eight rubella cases were detected in August (n=1), November (n=1) and December 2013 (n=3) through February 2014 (n=3). Laboratory QA is in place.

Surveillance for congenital rubella syndrome (CRS) is also conducted in southern Viet Nam. In 2013, 355 cases were reported from three hospitals. Of these, only three (<1%) were rubella positive. Among 104 cases reported in 2014 as of April, only 2 (1.9%) were rubella positive. The laboratory also performed virus isolation from 2013 to April 2014 and of 240 throat swabs samples received and processed, 88 (36.7%) measles viruses were isolated. Measles genotype identified in 2013 was D8, and in 2014 as of April, D8 and H1 genotypes were identified. Due to the outbreak, shortage of measles and rubella testing kits is a concern.

## **2.9.2 Polio Network Laboratories**

### *Australia*

Dr Bruce Thorley reported on Australia's AFP surveillance, which was established in 1995 and is coordinated by VIDRL and the Australian Paediatric Surveillance Unit. Paediatricians submit a clinical questionnaire and the case's medical history is collected by the Paediatric Active Enhanced Disease Surveillance (PAEDS). The National Polio Reference Laboratory was established at VIDRL in 1994, designated as National Enterovirus Reference Laboratory in 2011. From 1995 to 2013, the sensitivity of non-polio AFP in meeting the global goal has been variable but Australia has met the minimum of 1 per 100 000 per year for the past six years.

The AFP surveillance performance indicator of collecting two stools in less than 14 days of onset of paralysis from 80% non-polio AFP cases has never been met since surveillance started, with the best rate of 55% in 2007. In 2013 N=9 EV71 viruses were detected from AFP cases, most of them with transverse myelitis.

As the Enterovirus Reference Laboratory for Australia, VIDRL has established a network of 12 laboratories in the country. The purpose is to survey enteroviruses in the country but also serves for the purpose of poliovirus surveillance.

Australia has implemented environmental surveillance (ES) as IPV has been used since November 2005 and all poliovirus detections are significant. Also there are gaps in AFP surveillance, especially in the stool collection rate. The target areas for environmental surveillance are: low vaccine coverage areas, humanitarian resettlement programmes, areas with a high frequency of long-term visitors from endemic regions, and universities with many foreign students. Australia experienced polio importation from Pakistan 2007 and reported a suspected compatible case in a person who recently visited Somalia in May 2014. The concentration method used for ES is the WHO method developed by National Public Health Institute (KTL), Finland. From 2010 to 2013, 36 samples were collected (12 per year, four each from three sites in NSW) and no polioviruses were detected. However, 81% of the samples were positive for enteroviruses (29 NPEV / 36 samples) by culture and 94% positive by pan-EV RT-PCR.

### *China*

Ms Wang Dongyan presented a summary of the Status of China Polio LabNet in 2013–2014. China's last indigenous WPV was in 1994. However, imported cases were detected in 1995, 1996, 1999 and 2011 with the importation of WPV1 into Xinjiang from Pakistan. Strong immunization campaigns in response to all importations mopped up the outbreak very quickly.

In 2013, China collected 5573 stool specimens from AFP cases that corresponded to 92.3% of all AFP cases detected. In 2014, January to May, 1837 stools samples were collected representing 79% of all AFP cases detected. Virus isolation from stools for 2013 showed NPEV rate of 10.6% and 5.9% in 2014 to May. Poliovirus detection rates were 1.8% and 1.6% respectively. There were 255 polio isolates from 162 cases or healthy children sent to NPL of China in 2013. Up until May 2014, 53 isolates isolated from 32 AFP cases or contacts, all of which had ITD and sequencing performed. The sources of stool samples collected from the provinces were: AFP cases, contacts of AFP, high risk floating populations, and healthy children survey.

Sequence results in 2013 of the 107 polioviruses detected identified 86 (80.4%) with <0.5% change in genome, seven (6.5%) were >0.5 and <1.0% and 14 isolates (13.1%) > 1.0% change from one AFP case of iVDPV. No viruses with greater than 0.5% change have been identified from the 14 isolates sequenced in 2014.

In 2013, China introduced the new algorithm in the China Polio laboratory network. Currently 23 provinces have the ability of testing real-time RT-PCR ITD and VDPV, and another 8 provinces without that capacity had their samples tested by the national polio laboratory. The quality of the China LabNet continues to be strong. The RRL in China CDC and all 30 provincial polio laboratories, except Tibet, passed the global virus isolation PT of 2012 sent by WHO in June 2013. During Aug-Sep, 2013, the RRL in China CDC and all 8 provincial polio laboratories assessed passed an on-site accreditation review by WHO experts with excellent performance.

On September 2012, the NPL and 23 trained provincial polio laboratories conducted an ITD and VDPV screening PT provided by CDC/WHO. All laboratories except Guizhou provincial polio laboratory have passed the PT with 100% score.

There are 30 provinces reporting cell sensitivity test results to NPL regularly with all results in the acceptable range for LQC titre. The Tibet Polio laboratory has recently re-established their LQC standard and cell bank, due to problems with the regular supply of liquid nitrogen.

A workshop in 2013 for the China Polio laboratory network, recommended carrying out routine mycoplasma detection in provincial polio laboratories. Methods and proficiency were sent to all laboratories and most have introduced testing. Three training courses on real-time PCR for poliovirus identification were held in China CDC national polio laboratory for participants from all 30 provinces with facilitators from US CDC and VIDRL RRL.

China has a strategy for VDPV of early detection leading to early response and early interruption. Two VDPVs were detected in 2013 and 2014. One type 2 from Guangdong (2013) and one recombinant type 2 and type 3 in Jiangxi identified as iVDPV with isolates detected in 2013 and 2014. Environmental Surveillance of Polioviruses in China is also carried out in 10 provinces since 2008.

In summary, China is concerned about the importation of WPV, especially from the polio endemic countries bordering China. Poliovirus isolation rates from AFP cases are relatively low, especially compared with past isolation rates. Laboratory staff change frequently and some laboratories do not have enough staff, and / or new staff need training. Some laboratories require the replacement of equipment.

### *Japan*

Dr Hiro Shimizu updated on the polio status of Japan. Sabin IPV was introduced in the routine immunization programme in September 2012. One stand-alone Sabin IPV (Sanofi) product is available and there are four domestic producers of diphtheria, pertussis (whooping cough), and tetanus (DPT)-Sabin IPV.

Japan identified an emerging immunity gap in OPV coverage in 2011–2012. This was in part due to public health concerns about the vaccine-associated paralytic poliomyelitis (VAPP) risk, recent VAPP cases (recipient and contact), and growing public recognition of the need for use of IPV in Japan. A lower routine OPV coverage, 2011–2012 encouraged a catch-up immunization of unimmunized children after IPV introduction in September 2012.

With the risk of the immunity gaps in Japan, an intensified polio surveillance strategy has begun which includes looking for polioviruses from: suspected polio cases, confirmed polio case (Category II Reportable Infectious Diseases including VAPP), poliovirus isolates under the routine infectious agent surveillance, national epidemiological surveillance of vaccine-preventable diseases, seroprevalence survey initiated, enterovirus (poliovirus) survey of stool samples from healthy children, environmental surveillance (from 2013) and the development of a Polio Outbreak Response Plan which is a preparedness plan for detection of and response to WPV importation and circulating VDPV in Japan.

The environmental surveillance was established in July 2013. The protocol has approximately 0.5 L of sample water collected once a month at inlet of sewage treatment, which covers a population of 100 000–300 000. After water concentration, virus isolation and identification using concentrate is performed at local public health institute. The period of survey is approximately 5-6 months/ site. If poliovirus is isolated, then, virus should be shipped to the NIID for ITD, and epidemiological background will be notified to Infectious Disease Surveillance Center (IDSC), NIID for further analysis.

Japan also has an enterovirus surveillance programme which is categorised into three major categories: Category II (all cases to be promptly reported after diagnosis) which include acute poliomyelitis (including VAPP); Category IV (all cases to be promptly reported after diagnosis) including acute “unknown” encephalitis; and Category V (reported by sentinel hospitals and clinics) and include hand-foot-and-mouth disease (HFMD) (weekly report from paediatric disease sentinel), Herpangina (weekly report from paediatric disease sentinel), acute hemorrhagic conjunctivitis (weekly report from eye disease sentinel) and aseptic meningitis (weekly report from sentinel hospitals). Dr Shimizu reported that HFMD surveillance has detected two large outbreaks in Japan, in 2011 and 2013. Major causes of HFMD in Japan are several enterovirus types including: CA6, EV71, CA16, HHV6 and HRV. A method NIID is working on to concentrate PVs using magnetic beads was described. This project is showing promise but some issues with declining titres still need to be addressed.

In a 2013 accreditation review by WHO, it was noted that the NIID polio laboratory had low staffing level to conduct the range of activities conducted by the GSL. The GSL was recommended to work with NIID management and WHO on solutions. The linkage between NIID and District Public Health Laboratories needs to be strengthened. The GSL's role is to ensure that first line diagnosis of AFP specimens is timely, accurate and quality-assured with reference to re-testing of 1 AFP case, and compliant with WHO standards. For the GSL – acting as a NL for Cambodia and the Lao People's Democratic Republic – it was recommended that NIID should: discontinue use of 24-well plates for virus isolation, finalize establishment of a new cell-bank, and solve temperature monitoring and other QA issues.

Cambodia shipped 135 stool samples in 2013 to NIID and the Lao People's Democratic Republic, 84 stools. Two Sabin PVs were detected from Cambodia and none from the Lao People's Democratic Republic. A possible contamination may have happened with samples from the Lao People's Democratic Republic and Cambodia as sequences of isolates from both countries were closely related, but not epidemiologically related. Investigation showed that contamination from adjacent wells in 24 well plates was the likely cause. NIID has subsequently moved to using tubes, as recommended by the WHO accreditation review.

#### *Hong Kong SAR (China)*

Dr. Janice Lo presented the status of polio and enterovirus surveillance in Hong Kong SAR (China). The NPL is involved in the diagnosis of enterovirus infections, AFP surveillance, enterovirus surveillance and serological surveillance. The NPL also carried out serological immunity surveillance every five years from 1990 with high immunity present. In 2007, IPV replaced OPV and in 2008 serological evidence supported high levels of immunity to IPV and comparable to OPV. The NPL performed well in its proficiency tests and has passed with a 100% score over the past four years for virological, ITD, sequencing and real-time panels.

#### *Malaysia*

Ms Rafidah Abdul Karim reported on polio surveillance in Malaysia. Malaysia has a population of 29 628 392 with approximately 9.5 million under 15 years. To reach the minimum rate of 1/100 000, 95 AFP cases are expected per year. The Institute for Medical Research (IMR) was designated as the sole National Poliomyelitis Laboratory in 1993 and in 1998 was fully accredited by WHO as an isolation and identification laboratory. In 2010, IMR was fully accredited as rRT-PCR for ITD and VDPV Laboratory.

Malaysia's history of polio eradication identified the last indigenous WPV cases in Perak in 1984. In 1992, Malaysia found three cases of imported WPV (2 laboratory confirmed and 1 clinically confirmed). In 2008, IPV was introduced into eight states and nationwide in 2010. Prior to 2012, fewer than 80% of cases had adequate samples, however, since 2012 >90% have been received. The laboratory also receives samples for HFMD, enteroviruses and meningoencephalitis and these samples make up approximately 10 times the number received for AFP. The NPEV rate for AFP has been approximately 4% to 5% over 2011–2013 but 13% to 14% for HFMD samples. The laboratory also reported a decline in the number of poliovirus isolates (Sabin) identified in the past four years with none reported during 2012–2014 after the introduction of IPV. The IMR has passed the global proficiency testing programme for isolation and ITD and real time PCR from 2010, however, 2013 PT results are still pending. Challenges include low NPEV isolation rate and are considering introducing another cell line and to use PCR to improve sensitivity; need another formal training for rRT-PCR; laboratory space constraints, however a new campus for IMR research activities is planned for 2016.

#### *Mongolia*

Dr Ichinkhorloo Bonduush reported on behalf of all the Polio laboratory staff. Mongolia has a population of 2.7 million with 797 642 (29.5%) under 15 years. Mongolia has developed a national preparedness plan for WPV importation, which was approved by Ministry of Health in

2014. The key requirement of the plan is to sustain high population immunity by routine and SIAs and by having a sensitive surveillance system for the detection of WPV importation.

Routine OPV coverage is high in Mongolia and has reached 98% for the past two years. All surveillance indicators for quality of AFP surveillance exceed the minimum requirements and the national EPI team collects AFP surveillance report from all provinces and capital city weekly with completeness of weekly AFP reporting from provinces reaching 97% overall in 2013.

Many provinces have a population below 100 000 with under-15 year population of less than 30 000. As such, Mongolia has established a healthy children surveillance programme to collect stool samples from 200–300 children per year.

Among 90 NPEV isolated from all sources, three were isolated from AFP cases, others were isolated from healthy and with other diagnosis children's case. From all 406 samples tested, 90 (22.1%) enteroviruses and 1 (0.2%) poliovirus Sabin strain were identified. The sensitivity of both RD and L20B cells meet the WHO requirements. Laboratory performance is high with the laboratory being fully accredited.

#### *New Zealand*

Ms Jacqueline Ralston presented the status of the National Poliovirus and Enterovirus Reference Laboratory, ESR, New Zealand. The last case of WPV in New Zealand was in 1977. The Ministry of Health has overall responsibility for Polio surveillance, immunization and laboratory activities. Paediatricians are required to report AFP cases to NZ Paediatric Surveillance Unit (NZPSU) immediately and all 163 paediatricians are required to provide monthly reports to NZPSU. The ESR Clinical Virology Laboratory is the National Polio Laboratory for New Zealand. The demographics of NZ are: NZ population: 4.4 million, with 848 000 children under 15 years, and an expected number of AFP cases of 8–9/year.

The NPL data for AFP cases over 2012–2014 (May) showed that no poliovirus was isolated. The NPEV rates for all clinical stool samples received were reported as: 37.5% in 2012, 34.5% in 2013 and 14.3% in 2014 (April).

A National Enterovirus Surveillance System has been established in New Zealand which is used to identify circulation patterns of enterovirus serotypes and determine predominant strains, describes clinical diseases associated with circulating serotypes, detects enterovirus outbreaks to assist public health intervention and supplements polio surveillance until eradication of WPV and cessation of global OPV use. Under the enterovirus surveillance system, an outbreak of EV68 was detected for the first time in New Zealand in 2010. EV68 is associated with respiratory illness and is more common in young children. Of the 15 samples isolated with EV 68, 11 (73%) are from children under 2 years. Enterovirus 74 was first identified in New Zealand in 2011 and isolated from a faecal sample from a 2 year old boy with AFP.

Cell sensitivity results were reported and showed a drop in the sensitivity of L20B cells for most of 2013 for all 3 types of PV. A 100% score in the polio PT panels over the periods 2010-2012 and are processing the 2013 panel.

#### *Philippines*

Ms Leonibel Reyes presented the status of the Department of Virology, Research Institute for Tropical Medicine (RITM), the National Polio Laboratory (NPL) for the Philippines. RITM has served as the Philippines' NPL under the Department of Health National Plan of Action for Poliomyelitis Eradication since 1991. The NPL regularly participates in EPI and social mobilization meetings, quarterly AFP Expert Panel Review for the classification of AFP cases, advocacy campaigns and orientation seminars for AFP surveillance. The laboratory receives

financial and technical assistance from WHO under the Global Polio Laboratory Network (GPLN) and from the Department of Health.

Over the past 15 years, the Philippines was not able to achieve the minimum 10% NPEV rate. The NPEV rate can be influenced by stool sample management, specimen shipment, and laboratory performance. However in 2013, the NPEV rate was 9.9%.

In 2013, 67% of cases had two adequate stool specimens collected one to two days apart and ≤14 days from onset, and 63% of stool samples reached the laboratory within 7 days (80% target). The NPL has performed at a high standard, testing 729 specimens last 2013 and in the first quarter of 2014 tested 133, accurately identifying polio through virus isolation and reporting more than 95% of results within 14 days of receipt at RITM in 2013. To facilitate the identification and characterization of polio isolates quickly, the NPL sent all of its isolates to the VIDRL RRL within 7 days in 2013 and NPL-PHL was permitted to begin ITD testing in 2014 after passing the necessary performance criteria. The NPL scored 100% for the most recent polio isolation proficiency test. The final results of the 2013 Proficiency Testing on 8 May 2014 were still awaited. The RD-A and L20B cell lines of the NPL are routinely checked for sensitivity, and all results are within the acceptable ranges.

Two polioviruses were identified in 2014 from throat swabs collected during collaborative influenza like illness (ILI) study with Tohoku University. ITD testing on these poliovirus isolates was performed by the NPL under its new designation and found to be Sabin-like PV1 and PV2.

Mycoplasma contamination of the working stock of RD and L20B cell lines has been a recurring problem for the NPL. The NPL has exerted efforts since 2012 to restrict access to the Virus Isolation and Cell Culture Laboratory to reduce the chances of mycoplasma contamination. Since 2013, all the cell lines tested were found to be free from Mycoplasma contamination.

Challenges include the implementation of new SOPs and Work Instructions as directed by the ISO 9001-2008 new format; training of back-up staff for the ITD/VDPV testing is needed; close monitoring of mycoplasma contamination of all cell cultures and NPEV isolation rate at the subnational level.

#### *Republic of Korea*

Dr Ji-Yeon Hyeon reported the status of the NPL at the Korea Centers for Disease Control and Prevention (KCDC). IPV was introduced and the estimated immunization rate is more than 95%. AFP surveillance in the Republic of Korea involves 50 Paediatric neurology hospitals and with an AFP enhancement research Project by Catholic University. The summary of the AFP surveillance showed that AFP cases detected increased from 76 expected AFP cases to 94 and 84 AFP cases during 2012 and 2013 respectively.

NPEV rates have varied over the years 2002–2013 with greater than 10% achieved only in years 2010–2013. NPEV rates were boosted by large outbreaks of EV71 in 2010–2013. Enterovirus surveillance system of KCDC uses real-time RT-PCR for detecting enteroviruses within 24 hours of preparation of specimen. The most common specimens received are faeces (32%), CSF (19%) and throat swabs (35%). In 2012 and 2013, EV 71 was the most commonly found enterovirus with EV30 the second most common.

#### *Singapore*

Dr Tsang Wing Sze reported on the strains of the National Polio Laboratory of Singapore in the Virology, Department of Pathology, Singapore General Hospital (SGH). The SGH provides laboratory diagnostic services for virus infections for patients from SGH and other hospitals, as well as clinics in Singapore serves as the national laboratory for poliovirus, measles and rubella virus. The NPL has met the accreditation standard of WHO since 1998.

The Singapore AFP surveillance system set up by Ministry of Health in 1995 with a requirement for the notification to Ministry of Health of all cases of AFP in children under 15 years and in 1996, notification to Ministry of Health of all patients at risk of diseases that could lead to AFP, whether or not AFP is present. At risk diagnoses include: poliomyelitis, all forms of encephalitis, myelitis, acute infective polyneuritis, mononeuritis, monoplegia and all AFP cases.

Other sources of samples used for surveillance for poliovirus include non-AFP stool specimens, non-stool specimens and environmental specimens. Environmental samples involve random sampling of raw or treated reservoir or river water, concentrated 10X, once/week. The rate of enterovirus detection in the past four years was reported to have declined

The SGH NPL has achieved 100% in all PT panels received 2010-2013, including those for virus isolation, rRT-PCR ITD and sequencing. Challenges include: decline in quantity of specimens received for enterovirus culture; at least 25 polio or non-polio enterovirus isolates are sequenced annually. The lack of a standardized protocol for sequencing VP1 of NPEV requires trial and error testing which leads to wastage of reagents.

#### *Viet Nam Ha Noi*

Dao Thi Hai Anh presented the status of the Enteroviruses Laboratory, National Institute of Hygiene and Epidemiology (NIHE), northern Viet Nam. NIHE has been a member of WHO Polio Laboratory Network since 1993 and has worked closely with national expanded programme on immunization (NEPI) to build a good surveillance system for polio and HFMD. WHO and CDC support provision of equipment and kits, reagents and media.

The procedure to identify poliovirus from AFP cases follows closely WHO LabNet recommended methods. For HFMD, NIHE uses rRT-PCR detection for EV71, semi nested RT-PCR and sequencing for genotypes of others EVs. RD cell line is also used for the isolation of EV71 and RT-PCR/sequencing technique for genogroup and sub-genogroup of EV71 from isolated samples.

All polioviruses detected were Sabin-derived and more than 90% were reported within 14 days. The new algorithm was introduced in 2011 and NIHE achieved a score of 100% for the 2012 new algorithm PT after gaining 95% in 2011.

For HFMD surveillance, Viet Nam had a large outbreak in 2010–2011 with 603/912 clinical samples from patients with hand, foot and mouth disease in 2011 collected from northern provinces of Viet Nam positive with enteroviruses. This included 275 positive samples with HEV71 (45.6%) and 328 positive samples with other enteroviruses (54.4%). In 2012, 603/912 HFMD cases (63.5%) were found positive for EVs by using enterovirus general primers, and snRT-PCR method. EV-71 with EVs detected by each method as 46% (275) and 54% (328) respectively. Enterovirus serotypes were confirmed by sequencing. Challenges include new laboratory staff with insufficient experience, and the need to train staff in molecular biology techniques such as: rRT-PCR, snRT-PCR, RT-PCR and sequencing.

#### *Viet Nam Ho Chi Minh City*

Dr Nguyen Thi Ngoc Phuong presented the status of the Laboratory of Enteroviruses, Pasteur Institute (PI), Ho Chi Minh City. The NPL in PI has been a member of the WHO Laboratory Network since 1992 and is responsible for the surveillance of Enteroviruses in the southern half of Viet Nam. The NPL completed the cell sensitivity test according to WHO Polio LabNet recommendations and achieved expected titres, which were reported to WHO within the required 48 hours.

## 2.10 Practical sessions

The hands-on training session was conducted in the laboratories of the Virology Division, PHLC, Centre for Health Protection, Hong Kong SAR (China). Participants were divided into six groups:

Group A: Singapore, New Zealand	Group D: Philippines
Group B: China	Group E: Republic of Korea
Group C: Malaysia, Mongolia	Group F: Viet Nam

The sessions were divided into polio and measles/rubella specific activities as well as common activities engaging both groups. Both groups performed cell culture and practiced cell counting, freezing and seeding of flasks and tubes, polio working with RD and L20B cells and measles/rubella group working on Vero/hSLAM cells. Polio and measles/rubella have different strategies for cell trypsinization in that polio requires laboratories to count cells and seed flask and tubes at a prescribed concentration, whereas measles/rubella, while not discouraging cell counting, permits laboratories to perform flasks to be split and seeded using a ratio of 1:2-1:5.

All participants took part in trypsinizing the appropriate cell lines and practiced counting according to the Polio Laboratory Manual method. Polio seeded RD and L20B cells according to the concentration method and measles/rubella participants seeded flasks according to the split ratio method. The Vero/hSLAM cells were very clumpy after trypsinization in most, but not all, participant's hands. Cells were also prepared for freezing in liquid nitrogen with the addition of DMSO. These prepared cryovials were then stored in a controlled rate freezer and stored at -70°C overnight.

In session 2, the polio group prepared and extracted stool samples and inoculated them into pre-prepared tubes. The measles/rubella group processed throat swabs and urine samples and inoculated them into pre-prepared Vero/hSLAM cells. The following observations discussed:

- The use of gas burners in the BSC is not recommended as it can create air turbulence and disturbs the air curtain, and can place the user at risk of infection. Flaming the tubes and flasks is not critical and the opening of these is within a "clean environment". Also to sterilize the necks of the plastic containers the temperature would need to reach 100°C which will melt the plastic and prevent proper closing of the vessel.
- When working in a BSC, it is recommended to minimize any restriction or interruption to the airflow. Work should be at least 10 cm from the front of the grill, in the middle of the cabinet. The airflow through the front, or any air grill should not be blocked and the number of items in the BSC should be kept to a minimum.
- For poliovirus stool sample preparation, media, glass beads and chloroform should be added to all tubes, all tubes recapped before adding the specimen to one tube at a time. Workers can use a tongue depressor or flat wooden stick to take an aliquot of the specimen for processing rather than the small round stick. It is important that laboratories are aware of the weight of the sample (2 grams of stool specimen in 10 ml is the recommended quantity). The use of 50 ml tubes with a wider opening is preferable to the small 15ml tubes.

In session 3 both groups prepared cells for freezing in liquid nitrogen. Following a lecture on cell sensitivity and authentication of cells lines, the polio group set up a poliovirus sensitivity test for both RD and L20B cells. The measles and rubella group observed this process as there is no requirement for establishing cell sensitivity due to virus isolation not being used for diagnostic purposes, but for monitoring molecular surveillance of chains of transmission. Cells inoculated

with extract samples were observed for CPE and all groups were able to detect early signs of CPE, both for poliovirus and for measles virus.

Session 4 included revival for observation of previously frozen cells. These cells were observed over the following two days and checked for viability. Virus cultures were observed and those showing advanced CPE passaged further for measles and passaged into the appropriate cell line according to the new algorithm for polio. The polio group observed and recorded their sensitivity plates.

Participants were provided with the methods and techniques for spotting virus extract onto Whatman FTA filter cards for stabilization of RNA and inactivation to allow safe transport to the reference or global specialized laboratory for molecular studies. It was stressed that the process should be carried out in the BSC and allowed to air dry within the BSC. Once dry the sample will be fully inactivated and it can be labelled and added to a resealable plastic bag with desiccant and shipped to the sequencing laboratory, or stored long term at -20°C.

Participants were taught validation of critical equipment: Thermometers / autoclave / Pipette / Biosafety cabinet/ ELISA reader and washer/ water bath. A demonstration of the calibration and correct use of thermometers was carried out and all participants provided with a calibrated thermometer to take back to their laboratories. Calibration of micropipettes using an automated microbalance was shown to the participants with an emphasis on the need for regular calibration to ensure accurate results. The difference between accuracy and precision was explained. Accuracy is the proximity of measurement results to the true value and precision is the repeatability, or reproducibility of the measurement.

In the final sessions participants observed their revived cells and change media, seeded cultures and passaged virus cultures. Polio participants observed and recorded results of cell sensitivity plates.

### **3. CONCLUSIONS**

#### **3.1 General**

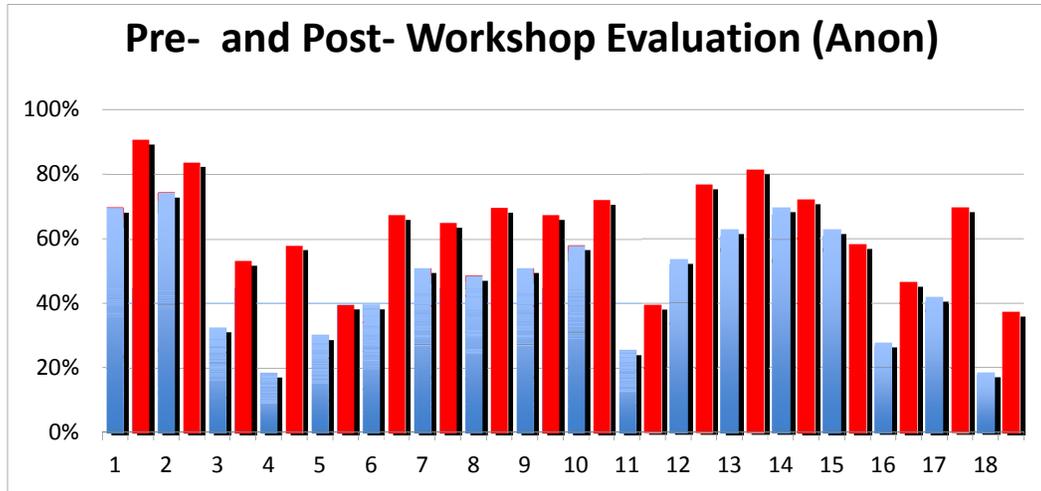
The training objectives were achieved. Participants gained technical knowledge and skills to maintain cell cultures for virus isolation based on WHO standard procedures, to perform virus isolation and identification using the new algorithm for polioviruses and the recommended standard procedures for measles and rubella viruses, and to perform cell sensitivity testing for polioviruses using WHO standard cell lines. The participants were also updated on the requirements for laboratory QA, including proficiency testing, WHO accreditation and data management and reporting.

#### **3.2 Evaluation of the workshop**

An assessment of the participants' knowledge of the measles/rubella and polio programmes was carried out pre- and post-training. This involved a 20 question quiz on: the eradication/elimination programmes, laboratory techniques and procedures, and the virology of all three diseases. The average score in the pre-training quiz was 46.5% (range 19%-74%) and the average score post-training was 63.8% (range 37%-91%) as shown in Figure 1. Quiz questions were discussed in an open forum to give participants opportunity to ask questions and address knowledge gaps.

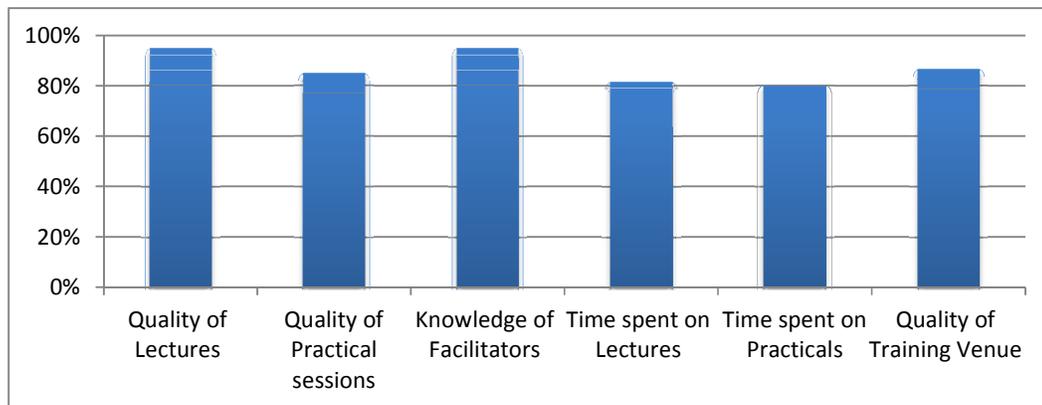
Figure 1. Anonymous results of the pre- and post-training evaluation for all participants.

The blue bar represents pre-workshop score and red bar represents post-workshop score, for all 18 participants.



An anonymous end-of-workshop assessment was also carried out. The results are presented in Figure 2.

Figure 2. Anonymous response to perceived quality of workshop (n = 12)





**Hands-on Training Workshop on Cell Culture Techniques  
for the Laboratory Diagnosis of Polio/Enteroviruses and  
Measles/Rubella Viruses in the Western Pacific Region  
Centre for Health Protection, Hong Kong SAR (China)  
19 to 23 May 2014**



Day	Time	Program				Facilitator
		Polio	Facilitator(s)	Measles		
Day 1	8:30	Registration				
	9:00 - 10:00	Inaugural session				
		Welcome				Director PHLC
		Introductions				Participants
		Overview of the WPRO laboratory network and Objective of the training				Youngmee Jee
		Overview of the global polio and measles situation				Youngmee Jee
		Pre training evaluation questionnaire				
	10:00	Photograph and Coffee break				
	10:30 - 15:00	Session 1	Cell Culture			
	10:30	Lecture 1	Overview of media for cell culture		Kai Cheung SZETO	
	10:50	Lecture 2A	Review of cell culture procedures (L20B and RD)		Bruce Thorley/Hiroyuki Shimizu	
	11:10	Lecture 2B	Review of cell culture procedures (VeroSLAM)		David Featherstone	
	11:20	Lecture 3A	Algorithm for specimen processing and virus isolation including stool specimen processing/inoculation(Polio)		Bruce Thorley/Hiroyuki Shimizu	
	11:35	Lecture 3B	Algorithm for specimen processing and virus isolation including specimen processing (TS and urine)/inoculation (measles)		David Featherstone	
	11:50	Lecture 4	Documentation / worksheets for cell culture and virus isolation (Polio)	Bruce Thorley/Hiroyuki Shimizu	Documentation / worksheets for cell culture and virus isolation (Measles)	David Featherstone
12:00	Walk through laboratory					
12:30	Lunch					
13:30	Practical 1	Cell culture: Cell count, freezing, seeding flasks/tubes	Participants/ Facilitators	Cell culture: Cell count, freezing, seeding flasks/tubes	Participants/ Facilitators	
	Practical 2	Observation and recording findings of preseeded RD and L20B tubes and flasks at varying concentration		Observation and recording findings of preseeded VeroSLAM flasks at varying concentration		
15:15	Coffee break					
15:30 - 17:00	Session 2	Virus isolation				
	Practical 3	Stool specimen processing/ inoculation - Polio group	Participants/ Facilitators	TS/ Urine /Oral fluid: Specimen processing/ inoculation - Measles group	Participants/ Facilitators	
17:00	Discussion on days work					
17:30	Adjourn					
Day 2	8:30 -12:30	Session 3	Cell sensitivity and authentication of cell lines			
	8:30	Lecture 5	Evaluation of cell sensitivity and authentication of cell lines		Bruce Thorley	
	9:00	Lecture 6	Worksheets and submission of cell sensitivity reports to WHO		Youngmee Jee	
	9:30	Coffee break				
	9:45	Practical 1 (continuation)	Cell culture: cell freezing into LN		Participants/ Facilitators	
	10:00	Practical 4	RD and L20B cell sensitivity test - Polio	Participants/ Facilitators	Observe RD and L20B cell sensitivity test for Polio	Participants/ Facilitators
	12:30	Lunch				
13:30 - 15:00	Session 5	Cell culture and virus isolation (Cont.)				

			Polio	Facilitator(s)	Measles	Facilitator	
	13:30	Practical 5	Observation of day 1 seeded culture: Tubes and flasks	Participants	Observation of day 1 seeded flasks	Participants	
			Observation and recording result of inoculated specimens on day 2 and passage of positive culture as per the algorithm		Observation and recording result of inoculated specimens on day 2		
			Observation and recording result of set of pre inoculated tubes /flasks		Observation and recording result of set of pre inoculated VeroSLAM flasks		
	14:30		Discussion on days practical and observation	Facilitators/Participants	Discussion on days practical and observation	Facilitators/Participants	
	15:00	Coffee break					
	15:30- 17:30	Session 6	<b>Presentation by participants (10 Mins each x 8 labs ) and discussion: Malaysia polio lab, Mongolia polio lab, New Zealand polio lab, Korea polio lab, Philippines polio lab, Singapore polio lab, Vietnam polio lab (NIHE and PI)</b>				
	17:30	Adjourn					
<b>Day 3</b>	8:30 - 9:30	Session 7	<b>Cell culture/virus isolation (Cont.)</b>				
		Practical 6	Revival of cells frozen on day 1	Participants/ Facilitators	Revival of cells frozen on day 1	Participants/ Facilitators	
			Observation of day 1 seeded culture: Tubes and flasks		Observation of day 1 seeded culture: Tubes and flasks		
			Observation of day 1 inoculated culture/freeze thaw and passage of positive cultures		Observation of day 1 inoculated culture/freeze thaw		
			Observation and recording result of set of pre inoculated tubes /flasks		Observation and recording result of set of pre inoculated tubes /flasks		
			Observation and recording results of cell sensitivity plates				
	9:30 - 10:40	Session 8	<b>Presentation by participants (10 Mins each x 7 labs ) and discussion: Malaysia measles lab, Mongolia measles lab, Korea measles lab, Philippines measles lab, Singapore NPHL, Vietnam measles lab (NIHE and PI),</b>				
	10:40	Coffee break					
	11:00 - 12:30	Session 9	<b>Shipment of specimens/isolates to RRL</b>				
	11:00	Lecture 7	SOP, packing and shipment requirements			Stanley Chan	
	11:15	Lecture 8	Use of FTA cards: Procedure for spotting and storage and extraction			Video Presentation	
	11:30	Practical 7	Use of FTA cards: spotting			Participants/Facilitators	
	12:30	Lunch					
	13:30 - 15:00	Session 9	<b>Good Laboratory Practices and quality assurance</b>				
	13:30	Lecture 9A	For Cell culture /Virus isolation / Molecular labs			Janice Lo	
	13:45	Lecture 9B	Standard Operating procedure (SOP)			Bruce Thorley/Hiroyuki Shimizu	
	14:00	Lecture 9C	Equipment maintenance and inventory			David Featherstone	
	14:15	Lecture 9D	Review of the Polio lab accreditation check list (Performance indicators)External quality assurance: Proficiency test	Bruce Thorley/ Youngmee Jee	Review of the Measles lab accreditation check list (Performance indicators)External quality assurance: Proficiency test/confirmatory tests	David Featherstone /Youngmee Jee	
			Discussion		Discussion		
	15:00	Coffee break					
	15:30 - 17:30	Practical 8	Demonstration of validation of critical equipment: Thermometers / autoclave / Pipette / Biosafety cabinet/ Elisa reader and washer/ water bath			PHLC Staff/David Featherstone	
	17:30	Adjourn					

			Polio	Facilitator(s)	Measles	Facilitator	
<b>Day 4</b>	<b>8:30 - 10:30</b>	<b>Session 10</b>	<b>Cell culture/virus isolation (Cont.)</b>				
	8:30	Lecture 10	Intratyptic differentiation of polio virus: wild/Vaccine/VDPV and reporting	Hiroyuki Shimizu/Bruce Thorley	Molecular genotyping of measles and rubella isolates and reporting	David Featherstone	
	8:45 - 9:30	Practical 9	Observation of revived cells and media change	Participants/ Facilitators	Observation of revived cells and media change	Participants/ Facilitators	
			Observation of day 1 seeded culture: Tubes and flasks		Observation of day 1 seeded culture: Tubes and flasks		
			Observation of day 1 inoculated culture/freeze thaw and passage of positive cultures		Observation of day 1 inoculated culture/freeze thaw		
			Observation and recording result of set of pre inoculated tubes /flasks		Observation and recording result of set of pre inoculated tubes /flasks		
			Observation and recording results of cell sensitivity plates		Observation of prepared tubes		
	<b>9:30 - 10:30</b>	<b>Session 11</b>	<b>Presentation by Participants (4 labs) : China polio lab, Japan polio lab, Australia polio lab, China measles lab</b>				
	10:30	Coffee Break					
	<b>11:00 - 13:30</b>	<b>Session 12</b>	<b>Biorisk Management training</b>			Bruce Thorley/Youngmee Jee	
	13:30	Lunch					
	14:30 - 17:00	<b>Session 13</b>	<b>Open Discussion on cell culture</b>			Facilitators/Participants	
	15:30	Coffee break					
	16:00	<b>Session 13</b>	Discussion continued				
	17:00	<b>Adjourn</b>					
<b>Day 5</b>	<b>8:30 - 10:00</b>	<b>Session 14</b>	<b>Cell culture/virus isolation (Final observation and reporting)</b>				
		Practical 10	Observation of revived cells	Participants/ Facilitators	Observation of revived cells	Participants/Facilitators	
			Observation of day 1 seeded culture: Tubes and flasks		Observation of day 1 seeded culture: Tubes and flasks		
			Observation of day 1 inoculated culture/freeze thaw and passage of positive cultures		Observation of day 1 inoculated culture/freeze thaw		
			Observation and recording result of set of pre inoculated tubes /flasks		Observation and recording result of set of pre inoculated tubes /flasks		
			Observation and recording results of cell sensitivity plates		Prepare cells from the tubes (cell smear) for immunofluorescence (measles and rubella)		
	10:00	Coffee Break					
	10:30 - 11:30	<b>Session 14</b>	<b>Post training evaluation</b>				
	<b>11:30 - 13:00</b>	<b>Session 15</b>	<b>Consolidation of results for presentation by group</b>			Participants	
			Presentation by Polio Groups (10 mins x 4 grps)			Participants	
			Presentation by Measles groups (10 mins x 4 grps)				
	13:00	Lunch					
	14:00-15:30	<b>Session 16</b>	Discussion and analysis of evaluation			Facilitators/Participants	
	15:30	Coffee Break					
	<b>16:00</b>	<b>Closing Ceremony</b>					

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