Meeting Report

Third Meeting on Vaccine Preventable Diseases Laboratory Networks in the Western Pacific Region

Manila, Philippines
5–9 September 2011
REPORT
THE THIRD MEETING ON VACCINE PREVENTABLE DISEASES LABORATORY NETWORKS IN THE WESTERN PACIFIC REGION
Manila, Philippines
5-9 September 2011

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NOTE

The views expressed in this report are those of the participants of the Second Meeting on Vaccine Preventable Diseases Laboratory Networks in the Western Pacific Region and do not necessarily reflect the policies of the World Health Organization.

This report has been printed by the Regional Office for the Western Pacific of the World Health Organization for the participants of the The Third Meeting on Vaccine Preventable Diseases Laboratory Networks in the Western Pacific Region, which was held in Manila, Philippines, 5-9 September 2011.
SUMMARY

The Third Meeting on Vaccine-Preventable Diseases Laboratory Networks in the Western Pacific Region was organized in Manila, the Philippines from 5 to 9 September 2011 to review the performances and identify the challenges of polio and measles/rubella network laboratories in the Region. To enhance biosafety awareness among polio network laboratories and measles/rubella laboratories, a biosafety session was jointly held for one and a half days during the week. The meeting reviewed ways to further strengthen the quality of the performances of network laboratories and also to monitor the implementation of recommendations from the second laboratory network meeting held in February 2010.

Around 75 participants from network laboratories, advisers and WHO staff were involved in the meeting. Participants included 55 country representatives from 16 countries (12 polio laboratories and 18 measles/rubella network laboratories), two WHO laboratory coordinators from Headquarters, three advisers from the United States Centers for Disease Control and Prevention (US CDC), two observers from Taipei (China) and selected WHO country EPI or laboratory focal points. The meeting also provided an opportunity to discuss the recent imported wild poliovirus cases in China and to further improve the timeliness of laboratory testing among network laboratories including subnational laboratories in China.

The objectives of the meeting were:

(1) to review the performance and the implementation status of new requirements of the polio network laboratories, identify challenges and define and develop plans for the expanding roles of the polio network laboratories to maintain polio-free status in the Region;

(2) to review the progress and identify challenges of measles/rubella laboratories and develop plans to further strengthen the performance of network laboratories including molecular surveillance; and

(3) to raise awareness regarding biosafety and security issues and establish or strengthen biosafety programmes among VPD network laboratories by conducting training for trainers, disseminating training modules and planning the roll-out of the biosafety training programmes.

Session I. Polio Laboratory Network

A session for regional polio laboratory networks was organized to discuss global progress towards polio eradication, to identify challenges in maintaining polio-free status in the Western Pacific Region, to share updates on global and regional polio laboratory networks, and to review the performance and implementation of new requirements of the polio network laboratories. The session included presentations on the detection of vaccine-derived polio viruses (VDPVs), experiences with real-time polymerase chain reaction (PCR) and sequencing among intratypic differentiation (ITD) laboratories, laboratory quality assurance, detection of polioviruses from non-AFP cases, experiences of the polio laboratory network for the laboratory diagnosis of hand, foot and mouth disease, data management and the country reports.
The meeting concluded that the performance of the regional polio laboratory network is sustained at polio-free certification standard and that acute flaccid paralysis (AFP) surveillance activities are efficiently supported by the polio laboratory network. The network laboratories provided critical evidence in support of the continued polio-free status of the Region. All network laboratories except China implemented the new algorithm for virus isolation. Being a polio-free region for more than 10 years, network laboratories in the Region have been actively involved in supplementary enterovirus or environmental surveillance. In particular, China established a very extensive hand, foot and mouth disease laboratory network based on existing polio and measles/rubella laboratories. Some countries in the Region (e.g. Australia, China, Japan, Malaysia and the Philippines) are either conducting or are interested in environmental surveillance to supplement AFP or enterovirus surveillance. Network laboratories are involved in those activities. Since the change of definition of type 2 VDPV during the global polio laboratory network meeting in 2010, China detected more type 2 VDPVs. The timeliness of sharing the laboratory results of VDPV detection with WHO has been improved in 2010–2011.

China expanded environmental surveillance in several provinces after organizing the third workshop in August 2010. Australia also introduced environmental surveillance, and Malaysia is planning to conduct environmental surveillance this year. The network continued to discuss the role further during the sessions on detection of polioviruses from non-AFP cases and on sharing experience of polio network laboratories for the laboratory diagnosis of hand, foot and mouth diseases. The establishment of environmental and enterovirus surveillance in a number of countries and areas in the Region has provided valuable data to support the maintenance of polio-free status of the Region. These virological surveillance systems can supplement AFP surveillance.

On 26 August 2011, China CDC confirmed four wild poliovirus type 1 strains, which are closely related to viruses circulating in Pakistan, through collaboration with the WHO global specialized laboratory in US CDC and the Pakistan National Institute of Health (NIH) laboratory. This event clearly shows the important role of the polio laboratory network and how quickly it can provide critical information to the national programmes and WHO. All polio network laboratories in this Region should be prepared to detect polioviruses with the highest efficiency in case of importation.

Session II. Biosafety training

Laboratory safety is of utmost importance to the Global Polio Laboratory Network (GPLN). The biosafety training session, which used six modules developed by GPLN, was held for one and a half days on 5–7 September. Meeting participants from both the polio and measles/rubella sessions joined the training and were divided into three groups. Trainers and rapporteurs were selected from the network laboratories. Each group had trainers for each module and two rapporteurs/presenters. The biosafety training was designed to share important messages and good practices, stimulate discussion and promote class participation, allow participants an opportunity to share opinions and experiences on safe working in the laboratory, and identify areas for improvement so that work can be performed more safely and the risk of release of poliovirus or other infectious pathogens to the environment can be minimized. At the end of the training, rapporteurs from each group presented a summary of discussions during the training.

Session III. Measles and rubella laboratory network

A two-day session of the regional measles and rubella laboratory network was organized to review the progress and identify challenges of the measles/rubella network laboratories and develop plans to further strengthen the performance of network laboratories including molecular
capacity in support of measles elimination and rubella control. The session included presentations on global and regional measles elimination and rubella control initiatives, quality assurance, measles outbreaks and laboratory performance, enhancing molecular surveillance, use of alternative sampling methods, strengthening rubella virus surveillance and the role of the laboratory, measles serosurvey, data management and reporting, laboratory diagnosis of mumps outbreaks in 2011 and country reports.

The meeting concluded that measles and rubella network laboratories provided high-quality support to achieve the regional goal of measles elimination by 2012 by confirming suspected cases and identifying measles virus genotypes circulating in the Region. The network consists of one global specialized laboratory in Japan, three regional reference laboratories (RRLs) in Australia, China and Hong Kong (China), 13 fully functional national measles/rubella laboratories (NMLs) and 31 provincial and 331 prefectural laboratories in China. Among 48 laboratories that are accredited by WHO, 47 laboratories were fully accredited as of August 2011. All provincial laboratories in China including Tibet laboratory, which was assessed in August 2010, have been reviewed and accredited.

As the role of the measles and rubella laboratory network also extends to molecular surveillance, laboratories with virus isolation, molecular diagnosis and sequencing capabilities are encouraged to perform virus isolation, sequencing and genotyping. Genotype and sequencing information are submitted to the WHO genotype and MeaNS database by national or regional laboratories. Genotype data on recent measles virus strains are available from all countries except Pacific island countries. In 2010–2011, Hong Kong (China) RRL continued to provide excellent support to identify genotypes of measles viruses circulating in Cambodia, the Lao People's Democratic Republic, Macao (China), Malaysia, Mongolia, the Philippines and Vietnam using confirmatory serum or virus isolation samples.

Laboratories with the capacity to conduct virus isolation and molecular detection were further trained during the follow-up training in November 2010 in Hong Kong (China). After the training, more network laboratories have become involved in virus isolation and molecular detection of measles and rubella viruses.

As of August 2011, 15 out of 17 laboratories that are supposed to send monthly laboratory data to the WHO Regional Office are sharing laboratory data on a monthly basis. Laboratory data including measles genotypes identified from each country have been included in the *Measles and Rubella Bulletin* of the WHO Regional Office since 2011.

The network should continue to make full efforts to obtain genotype and sequence information on measles and rubella viruses circulating in the Region. The laboratories should regularly communicate and collaborate with the national surveillance or epidemiology groups and the WHO Regional Office to minimize discrepancy of laboratory and surveillance data, and the delay in testing of samples and regular reporting of laboratory data to the WHO Regional Office.
1. INTRODUCTION

1.1 Background information

The polio and measles/rubella laboratory networks have played a crucial role in maintaining the polio-free status of the Western Pacific Region and in making progress towards the regional goal of measles elimination by 2012 by providing timely and reliable laboratory confirmation and virus identification. The performance of the polio and measles/rubella network laboratories has been monitored through a well-established WHO accreditation system. As of May 2011, all 43 poliomyelitis network laboratories and 47 out of 48 measles and rubella network laboratories in the Region, including subnational laboratories in China, have been fully accredited by WHO.

Based on observations during on-site accreditation exercises, important gaps in biosafety and security (BSS) were frequently noticed in many laboratories of the Global Poliomyelitis Laboratory Network (GPLN) and other vaccine-preventable disease (VPD) network laboratories. To address these concerns, GPLN developed audiovisual training materials presented as six modules with specific themes. By engaging in the setting up of BSS programmes, members of laboratory networks can help national authorities to control the use of biological materials.

1.1.1 Global Poliomyelitis Laboratory Network

GPLN, which comprises global specialized, regional reference, national and subnational laboratories, plays a crucial role in the global polio eradication initiative. The polio laboratory network in the Western Pacific Region consists of one global specialized laboratory (GSL) in Japan, two regional reference laboratories (RRLs) in Australia and China, and nine national polio laboratories (NPLs), including 31 provincial laboratories in China. In addition to the GSL and RRLs, four NPLs in the Region, namely, Hong Kong (China), Malaysia, New Zealand and Singapore, can conduct intratypic differentiation (ITD) of polioviruses.

The regional poliomyelitis laboratory network has played an important role in certifying poliomyelitis eradication in 2000 and maintaining polio-free status by providing accurate and timely laboratory results of acute flaccid paralysis (AFP) samples in the Region. During the wild poliovirus outbreak in Xinjiang, China following the importation of wild poliovirus type 1 from Pakistan in 2011, the polio laboratory network in China provided timely laboratory confirmation and identification of wild poliovirus to the programme for appropriate actions and responses.

Besides timely identification of wild polioviruses, the rapid detection of vaccine-derived poliovirus (VDPV) that causes AFP is becoming increasingly important because of polio outbreaks reported due to circulating VDPV. In this context, a new standard WHO algorithm for poliovirus isolation and identification has been introduced to the network. With the introduction of the new algorithm, all 12 NPLs, excluding 31 subnational laboratories in China, are using the new algorithm for virus isolation. Virus isolation results are reported within 14 days of receipt of the samples, and ITD results are reported within 7 days after virus isolation.

The performance of network laboratories is monitored through a laboratory accreditation programme established by WHO. Elements of WHO accreditation are proficiency testing, on-site performance reviews, monitoring of accuracy, and timeliness of reporting. All laboratories in the regional poliomyelitis laboratory network are performing at WHO accreditation standards.
Despite continued good performance of the polio laboratory network in the Region, concern has been expressed about the challenges of maintaining certification standards for reporting and investigating AFP cases and collecting adequate stool specimens. There is concern that priorities may have been directed to other public health activities after certification and that complacency may have evolved following the interruption of wild poliovirus transmission in the Region.

Rapid confirmation of the transmission of wild polioviruses and VDPVs is an essential first step towards implementing interventions to prevent virus spread. GPLN has formulated a strategic plan to reduce laboratory reporting time without compromising poliovirus detection sensitivity by introducing the new algorithm for virus isolation and ITD of polioviruses. In the Western Pacific Region, the new test algorithm for virus isolation was introduced among network laboratories (not including China’s laboratories) during the period 2009–2010, and real-time polymerase chain reaction (PCR) for ITD of polioviruses and VDPVs was introduced among five polio laboratories with ITD function.

1.1.2 Biosafety training

Microbiological laboratories always face risks of laboratory-acquired infections. It is sometimes difficult to determine whether a discovered infection in a laboratory worker was caused by a micro-organism present in the laboratory or whether it was acquired in the community. In the context of emerging and re-emerging diseases, laboratory-acquired infections present a public health concern as an infected worker may represent the index case for transmission of infectious agents to his/her relatives and other members of the community. One can also imagine the potential threat posed by wild poliovirus isolates present in a laboratory of a country that has eliminated poliovirus transmission. To raise awareness among polio network laboratories of biosafety, including highly dangerous infectious materials, biosafety audiovisual training materials were developed. These materials were designed for easy distribution to all WHO regions with the following objectives:

(1) to encourage each laboratory to designate a safety focal point to be trained in biosafety and to subsequently conduct on-site biosafety training using the materials provided;

(2) to encourage discussion on the risks associated with work in each laboratory’s unique setting; and

3) to encourage the exchange of ideas and feedback on relevant biorisk topics among laboratory personnel.

By engaging in the setting up of a BSS programme, members of laboratory networks can play an important role in helping national authorities to fulfil their obligations regarding the control of biological materials.

The International Health Regulations (2005) defines the rights and obligations of countries to report public health events, and also requires countries to strengthen their existing capacities for public health surveillance and response. Furthermore, World Health Assembly resolution WHA58.29 calls for the "enhancement of laboratory biosafety". As WHO is working closely with countries and partners to provide technical guidance and support to mobilize the resources needed to implement these rules, the Third Meeting on Vaccine-Preventable Diseases Laboratory Networks in the Western Pacific Region will introduce biosafety training for network laboratories including polio and measles/rubella laboratories to build knowledge and capacities on BSS issues.
During this meeting, one and a half days of biosafety training was organized on Day 2 and Day 3 for the poliomyelitis and measles/rubella network laboratories.

1.1.3 Measles and Rubella Laboratory Network

The WHO Global Measles and Rubella Laboratory Network, which comprises global specialized, regional reference and national laboratories, was set up following the GPLN model. A formal accreditation system to ensure the quality of the network laboratories has been established.

The strength of the measles and rubella laboratory network is attributed to the standardization of all procedures and participation in the WHO accreditation system. Network laboratories use standardized methods of IgM assays, virus isolation and identification; participate in annual proficiency testing and confirmatory testing; participate in WHO-coordinated meetings, workshops and hands-on training courses; and report laboratory data to WHO on agreed intervals.

The WHO measles and rubella laboratory network has played a crucial role in the measles elimination initiative. Provision of timely and highly reliable laboratory confirmation and virus identification results to the programme is essential as we approach the goal of measles elimination. Establishing baseline genetic data on measles viruses that are currently circulating in the Region will allow us to differentiate importation and indigenous circulation of measles virus strains.

The measles and rubella laboratory network in the Western Pacific Region consists of one GSL, three RRLs, 16 national laboratories and 362 subnational laboratories in China. WHO conducts the accreditation of 48 network laboratories including 31 provincial laboratories in China. Among four designated laboratories in Pacific island countries, only one laboratory is fully functional, while two laboratories still participate in the WHO proficiency programme.

The WHO Western Pacific Region established the twin goals of measles elimination and hepatitis B control in 2003 and held the first regional laboratory meeting for the measles/rubella laboratory network in 2004. In 2005, the target year to achieve the twin goals was set as 2012. In July 2008, the Regional Measles and Rubella Laboratory Network Meeting was held as a part of 17th TAG meeting (first VPD laboratory network meeting).

Annual proficiency testing has been in place in the Western Pacific Region since 2001. In 2009, all network laboratories including 31 provincial laboratories in China participated in the same WHO proficiency-testing programme for the first time. As of December 2009, among 48 fully functional network laboratories including 31 provincial laboratories in China, 46 laboratories are fully accredited by WHO. In addition, 331 measles laboratories in prefectural Chinese Centers for Disease Control and Prevention (China CDC) are operated under the WHO standards.
1.2 Objectives

The objectives of the meeting were:

1. to review the performance and the implementation status of new requirements of the polio network laboratories, identify challenges and define and develop plans for the expanding roles of the polio network laboratories to maintain polio-free status in the Region;

2. to review the progress and identify challenges of measles/rubella laboratories and develop plans to further strengthen the performance of network laboratories including molecular surveillance; and

3. to raise awareness regarding biosafety and security issues and establish or strengthen biosafety programmes among VPD network laboratories by conducting a training for trainers, disseminating training modules and planning the roll-out of the biosafety training programmes.

1.3 Appointment of workshop officers

Dr Bruce Thorley was appointed as chairperson, Dr Hiroyuki Shimizu as vice-chairperson, and Dr Janice Lo as rapporteur for the polio laboratory network session and biosafety training.

Dr Paul Rota was appointed as chairperson, Drs Makoto Takeda and Xu Wenbo as vice-chairpersons and Ms Jennie Leydon as rapporteur for the measles and rubella laboratory network session.

1.4 Organization

The timetable of the meeting is provided in Annex 1. The list of participants is included in Annex 2. Table 1 provides a breakdown of attendees by country and session.

### Table 1. Participants of the Third Meeting on Vaccine-Preventable Diseases Laboratory Networks in the Western Pacific Region

<table>
<thead>
<tr>
<th>Country or area</th>
<th>Session I: Polio laboratory network</th>
<th>Session II: Biosafety training</th>
<th>Session III: Measles/rubella laboratory network</th>
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2. PROCEEDINGS

Session I: Poliomyelitis Laboratory Network was held for one and a half days from 5 to 6 September 2011 to review the performances of the poliomyelitis network, identify challenges of network laboratories and ways to strengthen the quality of the performances, and discuss the progress of implementation of the recommendations from the network's meeting in February 2010. Participants included 14 representatives from polio laboratories in 12 countries and areas, the polio laboratory coordinator from WHO Headquarters, and temporary advisers from the United States Centers for Disease Control and Prevention (US CDC).

Session II: Biosafety Training was convened from 6 to 7 September for one and half days for representatives from polio and measles/rubella laboratories as well as designated biosafety officers from national public health institutes.

Session III: Measles/Rubella Laboratory Networks, which took place from 8 to 9 September, was attended by 22 representatives from measles laboratories in 17 countries as well as temporary advisers from US CDC.

Observers from Research Institute for Tropical Medicine, Korea Centers for Disease Control and Prevention, Centers for Disease Control and Prevention in Taipei and Institute of Biomedical Sciences, Taipei also participated in the meeting.

Opening session

Dr Sergey Diorditsa, Team Leader of the Expanded Programme on Immunization (EPI), welcomed all country participants and advisers from partner organizations. The Director of Programme Management, Dr Hans Troedesson, opened the meeting. He acknowledged the close collaboration of polio and measles/rubella network laboratories with national EPI and surveillance programmes in each Member State, which has greatly contributed to maintaining polio-free status and making progress towards measles elimination in this Region.
2.1  Polio Laboratory Network

2.1.1 Overview of the Global Polio Eradication Initiative (GPEI) and maintenance of poliomyelitis-free status in the Western Pacific Region

(1) GPEI and regional updates on maintaining poliomyelitis-free status

Dr Sigrun Roesel presented an overview of the current status of GPEI and maintenance of the polio-free status in the Region. She noted polio virus transmission has never been stopped in four countries, polio virus importation has occurred in some polio-free countries and re-established transmissions following importation have been observed in several countries. She also presented progress made on achieving milestones set by GPEI, as of June 2011. All poliovirus importations that occurred in 15 countries in 2009 were interrupted by mid-2010. Interruption of all new poliovirus importations within six months was achieved and on track. By the end of 2010, re-established transmissions in Angola, Chad, the Congo and Sudan had not been interrupted. While two of four polio-endemic countries, India and Afghanistan, are on track for interruption by the end of 2011, Nigeria is still at risk and Pakistan is off track. Wild poliovirus type 1 was predominant in Western African countries, where new infections were re-established in 2010 and the circulation of type 3 wild poliovirus strains after importation was observed in 2011. In India, after type 2 wild poliovirus was eradicated in 1999, type 1 became predominant, but cases have been decreasing after the introduction of monovalent oral polio vaccine (OPV) in 2005. With the introduction of bivalent OPV in 2010 in India, circulation of both type 3 and type 1 wild poliovirus strains decreased. Last reports of type 3 and type 1 polio cases in India were made in October 2010 and January 2011, respectively. Rapid and intense mop-up campaigns have been conducted, and an emergency preparedness and response plan was prepared and shared with state governments in India in 2011.

The status of poliomyelitis in the Region was also presented. Sixteen out of 37 countries and areas are using inactivated polio vaccine (IPV), and 22 out of 37 countries and areas have a booster-dose schedule. In 2008, there were six countries with OPV coverage below 90%, namely, the Philippines (80%–90%), Kiribati, Samoa, Solomon Islands (70%–80%), the Lao People’s Democratic Republic and Papua New Guinea (60%–70%). In 2010, 10 countries and areas had OPV coverage below 90%. Most notably, the Lao People’s Democratic Republic, Papua New Guinea, the Philippines and Solomon Islands had OPV coverage of 70%–80%. At the subnational level, the proportion of districts with <80% DPT3 coverage in 2010, which is a proxy indicator of OPV3 coverage, was 12% in Cambodia, 70% in the Lao People’s Democratic Republic, 14% in Malaysia, 66% in Papua New Guinea, and 27% in the Philippines. These figures may not reflect subnational susceptibility, and identifying underserved populations such as migrants, minorities and slum dwellers would be critical. During the period 2009–2011, Papua New Guinea kept low non-polio AFP rates of around 0.5, and Cambodia and the Philippines had non-polio AFP rates of <1.0 in 2011 (as of August 2011).

Risk assessment was conducted in the Region, and supplementary immunization activities (SIAs) were conducted in Cambodia, the Lao People’s Democratic Republic and Papua New Guinea. In Cambodia, two rounds of OPV SIAs in high-risk communities will be implemented in combination with measles SIAs. In the Lao People’s Democratic Republic, two rounds of phased nationwide OPV SIAs were completed in combination with Child Health Days and tetanus-toxoid (TT) SIAs. In Papua New Guinea, OPV was added to the nationwide phased measles SIA and will be added again in 2012. In China, two rounds of OPV SIAs in high-risk areas, environmental surveillance, healthy children stool surveys, serosurveys and training on risk assessment will be conducted. In Malaysia, OPV SIA in unregistered populations in Sabah (Borneo) will be considered. In the Philippines, OPV was added to TT SIAs in high-risk areas. In
Viet Nam, two rounds OPV SIAs in high-risk areas based on subnational risk assessment will be conducted, possibly combined with TT.

For sharing country experiences and updating information, an international polio workshop was held in Urumqi, China in July 2011. The workshop was attended by representatives from 12 countries, four WHO regional offices, partner agencies such as the United States Agency for International Development (USAID) and US CDC, as well as experts from China CDC and provincial CDCs.

The detection of wild poliovirus type 1 from four cases in Hetian Prefecture, Xinjiang Uygur Autonomous Region, China in August 2011 was also mentioned. Two rounds of OPV SIAs will be conducted from 8 to 12 September and 8 to 12 October 2011.

(2) Status of Global Poliomyelitis Laboratory Network

Dr Ousmane Diop from WHO Headquarters presented an update on wild poliovirus detection, global distribution and transmission links, GPLN performances, quality assurance programme and new developments in GPLN. Hot spots of wild poliovirus cases in 2010 were presented, including: four European countries with new wild poliovirus type 1 cases, three African countries with re-established transmission, and two African countries with new wild poliovirus type 1 outbreaks. Approximately 1% of specimens from 1293 cases yielded wild polioviruses, with a type 1 to type 3 ratio of 14:1. Ninety-two per cent of the workload was from AFP samples and 8% was from non-AFP sources. Genetic analysis showed that following the wild poliovirus outbreak in Tajikistan in 2011, there were at least eight separate importations of wild poliovirus into the Russian Federation and Turkmenistan. More than 80% of virus isolation results were reported on time from five regions, except the European Region in 2010. In five regions, except the African Region, more than 80% of ITD results were provided within 60 days of AFP onset. Overall, high-quality performance has been maintained in GPLN despite the increasing workload over the past 20 years. Referral of samples has been improved by using FTA cards for shipment. Real-time PCR for ITD and VDPV screening has been fully implemented in around 60 laboratories. Implementation of quality assurance for sequencing has seen good progress. Sequencing proficiency testing using the same panel as real-time reverse transcriptase polymerase chain reaction (rRT-PCR) PT started in 2011. Biosafety training materials consisting of six modules and training guidelines were developed and launched in 2010. The aim of the biosafety project is to improve implementation of biosafety practices and biorisk management in the laboratory. Advanced training on biorisk management will be held in November 2011. The main advantages of FTA cards for transportation of dried blood spots (DBS) were also presented, highlighting technical matters, timeliness, costs and regulatory issues.

(3) Regional updates of poliomyelitis laboratory network

Dr Youngmee Jee, regional laboratory coordinator, presented updates on the activities of the polio laboratory network in the Region from 2009 to 2011. By 2010, a new algorithm for virus isolation was fully implemented by all network laboratories in the Region except in China. There were 192 poliovirus isolates recorded in 2009 (China 176, Philippines 12, Viet Nam 3, Lao People’s Democratic Republic 1), 211 in 2010 (China 203, Philippines 4, Viet Nam 2, Cambodia 1, Papua New Guinea 1) and 92 in 2011 (China 89, Viet Nam 2, Brunei Darussalam 1). All 43 network laboratories including the Tibet CDC subnational polio laboratory were fully accredited, and seven ITD laboratories including a newly upgraded polio laboratory in Malaysia were accredited for ITD function. Five ITD laboratories in Australia, China, Japan, Malaysia and Singapore implemented real-time PCR in 2010, while NPLs in Hong Kong (China) and New Zealand still used conventional ITD PCR. ITD results from AFP and non-AFP cases were presented. In 2010, seven aVDPVs were detected in China: four aVDPVs from AFP (three type 2
and one type 3) and three aVDPVs from non-AFP (two type 2 and one type 3). In 2011, two aVDPVs and three iVDPVs were detected from China: three VDPVs from AFP (one type 1, one type 2, one type 3) and two VDPVs from non-AFP (both type 2). Timeliness of reporting ITD results has been improving, from 93% in 2009 to 97% in 2011. Wild poliovirus outbreaks in Tajikistan in 2010 and China in 2011 were presented, and the importance of rapid response including timely detection of poliovirus by polio laboratories was highlighted. All 43 polio laboratories passed virus isolation PT and seven ITD countries including China and Japan passed ITD with a 100% score in 2010. Some polio laboratories, such as those in Hong Kong (China), Japan, Malaysia, Mongolia, the Republic of Korea, Singapore and Viet Nam, have been involved in hand, foot and mouth disease (HFMD) diagnosis. Environmental surveillance was also conducted in China and Australia, and an iVDPV study was conducted in China and the Philippines. Most of the recommendations made during the second VPD laboratory network meeting were carried out. All network laboratories except China successfully switched to the new algorithm for virus isolation PT, and five ITD laboratories implemented real-time PCR for ITD and VDPV screening. Remaining challenges include timeliness of reporting poliovirus and shipping isolates within 7 days (14 days for China), maintenance of laboratory equipment, and monthly sharing of non-AFP data with WHO. Regarding the biosafety training, focal points are required to liaise with WHO and conduct training in each country. Plans for 2012, such as distribution of new reporting format, hands-on training and supporting environmental surveillance, were also discussed.

2.1.2 Detection of vaccine-derived polioviruses (VDPV)

(1) Global update on VDPV detection

Dr Ousmane Diop presented the global update on VDPV detection and monitoring. Regardless of immunological or clinical outcome, Sabin-like viruses are excreted by some vaccine recipients. Secondary transmission of Sabin-like viruses is known to occur among household and/or community contacts. However, in high-hygiene and low-susceptibility settings, no or limited secondary transmission of excreted Sabin-like viruses occurs. Replication of virus occurs both in recipients and contacts. Definitions were given for the three categories of VDPV: (i) iVDPV in persons with primary immunodeficiency condition; (ii) cVDPVs that circulate and cause outbreaks of poliomyelitis; and (iii) aVDPV, for which the origin of virus is ambiguous. The number of nucleotide substitutions of types 1 (>9), 2 (>5) and 3 (>9) VDPV strains was also explained.

In 2010, approximately 2.2 billion doses of polio vaccine including monovalent OPV type 1, monovalent OPV type 3, bivalent OPV and trivalent OPV were used for polio SIAs in all WHO regions except the Americas. Eighty-five VDPV cases were reported from 2010 to August 2011. Type 1 cVDPV was detected from Mozambique and type 2 cVDPD from Nigeria, Somalia and Yemen between February and August 2011. Data showed cVDPV outbreaks could be extensive and/or prolonged and indicated co-existence and/or persistence of multiple chains of transmission for several years. The majority of cVDPV cases (~60%) were under 3 years old, and around 60% of cVDPV cases received fewer than three doses. Investigation of contacts, risk assessments, timely immunization response and selection of adequate vaccine (monovalent, bivalent or trivalent OPV) are key responses to cVDPV detection. It should be noted that VDPVs are treated like wild polioviruses. Since 2008, detection of VDPVs has increased, which might indicate increased serotype-specific immunity gaps. The role of monovalent OPV in the emergence of cVDPV in some settings is not clear. GPLN has become the primary source of new VDPV detection. Inadequate polio immunization such as absence of corresponding wild virus type and low population immunity is the primary risk factor for VDPV emergence. Adequate VDPV detection from all sources and confirmation and reporting are important for effective control.
Dr Zhang Yong gave a presentation on VDPV surveillance in China in 2010 and 2011. During those two years, China witnessed an increase in the number of detected VDPV cases and the emergence of newly identified iVDPV and aVDPV. Based on the surveillance data from 2000 to 2009, about one or two VDPVs were found annually in China; however, seven VDPVs were identified in 2010 and 2011. With the new definition of type 2 VDPV, more type 2 VDPVs have been detected since 2010. VDPVs were found in some areas such as Ningxia and Tibet, where no polioviruses were detected from AFP cases in 2010. The clinical features of recent VDPV cases were also presented. Two of those cases had primary immunodeficiency with excreting VDPV, and one case had been excreting type 3 iVDPVs for more than six months. The current polio immunization strategy in China is effective in preventing sustained transmission of VDPVs. Young VDPVs with less than 10 nucleotide changes for types 1 and 3 or six nucleotide changes for type 2 have been detected and early actions have been taken in China. It was highlighted that transmission of VDPVs can be controlled by a high-quality surveillance programme that permits very early detection and response.

Massive OPV use may have paradoxically contributed to the difficulty of total global polio eradication due to vaccine-associated paralytic poliomyelitis (VAPP) and VDPV. OPV can replicate in the gut of immunocompetent persons for up to 40 days after administration and is prone to intratypic and intertypic recombinations leading to genetically divergent strains. Persons with primary immunodeficiency disorders (PIDD) who are exposed to polio through OPV vaccination or from contacts of vaccinees may become potential reservoirs of iVDPV due to prolonged excretion. Dr Marysia Recto presented prevalence of prolonged and chronic poliovirus excretion among persons with PIDD in the Philippines. PIDD patients, especially those with B-cell or humoral immunodeficiencies may become potential reservoirs of iVDPV due to prolonged excretion. Genetic evolution of iVPDV occurs faster and can arise any time in an immunodeficient patient despite high rates of vaccine coverage in the community. A cross-sectional study was conducted to estimate the prevalence of VDPV excretion among persons diagnosed with PIDD in the Philippines, to describe the clinical features of PIDD patients excreting poliovirus, to genetically characterize VDPV isolated from persons with PIDD, and to determine the duration of poliovirus excretion among subjects who tested positive for VDPV excretion. The subjects were 71 Filipino patients consisting of 21 confirmed PIDD cases and 50 probable PIDD cases under 35 years old. Initial screening was conducted with two visits including blood and stool sample collection. In the second phase, for follow-up of chronic iVDPV excreters, monthly stool virus isolation was implemented until two months of sequential negative poliovirus isolates. Prolonged excretion was defined as iVDPV > 0.5% divergence and repeated detection of iVDPV in the same patient for more than 6 months of stool collection. According to the shown results, no chronic excreters of iVDPV were detected among the Filipino patients with PIDD involved in this study. Although the majority of PIDD patients either received or were exposed to OPV, the risk of developing iVDPV was rare. One patient transiently excreted Sabin-like poliovirus type 1 six days after the third dose of OPV. Improved diagnosis of PIDD patients together with intensive surveillance may be necessary to assess further risks of chronic iVDPV infections in the general population.
poliovirus infection since the 1950s in Japan was shown. The last polio outbreaks in Japan occurred between 1960 and 1961. After the introduction of OPV in 1964, the incidence of polio declined drastically. Since 1981, no indigenous polio case has been detected and only VAPP cases have been detected in Japan. To maintain polio-free status in Japan, VAPP surveillance and infectious agent surveillance, healthy children stool surveys and serosurveys to detect polio neutralizing antibody titers were conducted. Age-specific OPV immunization history was analysed for each age group. To introduce IPV in Japan, a DPT-IPV development plan was initiated in 2002. Japan is expected to have DPT-IPV licensed by late 2011.

The NIID polio laboratory serves as NPL for Cambodia and the Lao People’s Democratic Republic and RRL for Mongolia, the Republic of Korea and Viet Nam. The Republic of Korea has not sent any isolates since the switch from OPV to IPV in 2005. Samples from 141 AFP cases in Cambodia and 119 AFP cases in the Lao People’s Democratic Republic were received between January 2009 and August 2011. Among those samples received from Cambodia and the Lao People’s Democratic Republic, 85 and 40 polioviruses were isolated, respectively. Non-polio enterovirus (NPEV) isolation rates from these samples ranged from 20% to 30% in 2010–2011.

The new algorithm has been fully implemented since January 2010. In July 2010, the evaluation of rRT-PCR method in NIID was completed. NIID obtained a WHO PT score of 100% for real-time PCR for ITD and VDPV screening. From July 2010, four PV1-SL, five PV2-SL, three PV3-SL, three PV1-NSL, three PV2-NSL and two PV3-NSL were isolated from AFP and non-AFP samples. All the isolates were finally identified as OPV-related polioviruses by VP1 sequencing. A training course on laboratory diagnosis techniques for the control of VPD is conducted annually at NIID, and participants from China, Malaysia, the Philippines and Viet Nam were invited to the January 2011 training. The Asian Environmental Surveillance Workshop was also held in July 2011, and international participants from China, Malaysia and Thailand were invited. Target diseases of the Infectious Diseases Control Law in Japan were revised on 1 February 2011, and the guideline of “Regulations on the safety control of labs handling pathogenic agents, NIID” was revised in 2010.

(2) ITD PCR and sequencing summary report, Australia, 2010–2011

Mr Jason Roberts from the Victorian Infectious Diseases Reference Laboratory (VIDRL) presented Australia’s ITD PCR and sequencing summary report for 2010–2011. ITD tests were conducted with 35 samples from the Western Pacific Region: Philippines (n=21), Malaysia (n=10), Papua New Guinea (n=2) and Brunei Darussalam (n=2). Twenty-one samples produced Sabin-like results and 14 produced discordant results from ITD testing. Sequencing was conducted for 23 cases, including 18 AFP and 5 non-AFP cases. Six PV1, nine PV2 and eight PV3 were detected. Sequence identities were as follows: 99.0%–100% for PV1, 99.7%–100% for PV2 and 99.8%–99.9% for PV3.

(3) Experience with real-time PCR and sequencing in China

Dr Yong Zhang presented experiences with real-time PCR for ITD and VDPV screening and sequencing in China. From January to June 2011, as a supplementary method to sequencing ITD method, 106 poliovirus isolates including five VDPVs were tested for ITD and VDPV screening using the real-time PCR method developed by US CDC. All 106 isolates had been sequenced after neutralization test. All 106 isolates produced consistent results for ITD, including 39 type 1, 34 type 2 and 33 type 3 strains. VDPV screening assays produced some false results. Three out of 39 (7.6%) type 1 VDPV strains, 22/34 (64.7%) type 2 strains and 22/33 (6.1%) type 3 strains were falsely flagged for further sequencing. Among 106 poliovirus isolates, the one type 3 strain with 18 nucleotide substitutions was not detected, while all type 1 and 2
strains were detected with VDPV screening assays. China CDC will continue to use VP1 sequencing as a primary ITD method and will use real-time PCR as a supplementary method for ITD and VDPV screening in China. These data help to improve to design the new primers and probes.

2.1.4 Laboratory quality assurance

(1) Report on proficiency testing: virus isolation

Dr Bruce Thorley from the WHO Polio Regional Reference Laboratory in VIDRL, Australia reported on the results of 2010 WHO PT, which was the first to use the new virus isolation algorithm. All laboratories reported their results within 14 days, and all reports were correct for the 10 samples. Results were reported as L20B positive, NPEV or negative. Additional passage of isolates of RD arm from RD+/L20B+ to RD+/L20B+/RD+ seemed to be an issue for some laboratories. The method of scoring results of PT for isolation polioviruses according to the new algorithm was presented. It was explained that 20 points are deducted from the score when polio-negative samples are contaminated with poliovirus or when poliovirus is missed. A passing score is 90%. VIDRL tested 416 samples including 257 NPEVs in 2010-2011 and poliovirus was not detected. VIDRL received AFP samples from Brunei Darussalam, Papua New Guinea and Pacific island countries. VIDRL is also involved in enterovirus surveillance and environmental surveillance.

(2) Proficiency testing for polio molecular diagnostic methods

Dr Steve Oberste, Polio and Picornavirus Laboratory Branch, US CDC presented proficiency testing for polio molecular diagnostic methods. US CDC developed nucleic acid probe hybridization (NAPH), conventional ITD PCR and real-time PCR for ITD and VDPV screening. Proficiency testing consisting of 10 unknown samples with lyophilized in vitro RNA transcripts containing target sequences was also developed by US CDC. It includes poliovirus (both single and mixtures), NPEVs and negative samples, and results should be sent to US CDC within 7 days. A passing score is >90%, with 50% of the score based on final results and 50% based on correct data interpretation. All laboratories in the Western Pacific Region obtained a 100% score for PT.

(3) Laboratory quality assurance of China polio laboratory network

Dr Yong Zhang from NPL, China CDC presented the status of China’s polio laboratory network in 2010 and 2011, VDPV surveillance and environmental surveillance of polioviruses in China, and challenges faced by China’s polio laboratory network. The RRL in China CDC and all 31 provincial polio laboratories passed PT in 2010 and 2011. In August 2010, RRL in China CDC and 12 provincial polio laboratories passed WHO on-site accreditation with excellent performance. The new cell sensitivity standard for provincial polio laboratories was established. The L20B (p21) and RD-A (p229) cells with low passage numbers were sent to the 31 provincial polio laboratories, and new cell banks were established. Twenty-seven provinces report cell sensitivity test results to NPL regularly, and all of the results were in the acceptable range for LQC titer. The National Workshop for China Polio Laboratory Network, the National Workshop for AFP Surveillance for China Polio Laboratory Network and environmental surveillance training were held in 2010 and 2011. In 2010, 12 provinces attended an environmental surveillance training course, and all laboratories isolated the polioviruses and NPEVs from the sewage samples. A brief summary of VDPV surveillance in China was also presented. As of June 2011, six strains were isolated from the iVDPV cases, all of which had 18 nucleotide changes. Concerns for importation of wild poliovirus from other countries, requirement of staff training and sample transportation issues were highlighted.
(4) Monitoring of cell sensitivity testing

Dr Fem Paladin, Technical Officer (Laboratory), reported on cell sensitivity testing from March 2010 to August 2011. A review of cell sensitivity testing data from this period showed that there has been significant improvement in the implementation of cell sensitivity testing in the Western Pacific Region. However, it is important to analyse and correlate cell sensitivity testing results with NPEV isolation rates and field conditions. The report should be presented in trend charts with titration results to review test validity, and absolute passage numbers of cells should be always included. A recommendation on cell sensitivity testing made during the previous VPD laboratory networks meeting, i.e. to report results within 48 hours of testing completion, was emphasized again.

(5) Accreditation of sequencing laboratories

Dr Steve Oberste of the Polio and Picornavirus Laboratory Branch, US CDC presented on the accreditation of polio sequencing laboratories. GPLN has many years experience in laboratory accreditation for poliovirus isolation and ITD, but there are still challenges in the accreditation of sequencing laboratories. Sequencing has fewer quantifiable variables than virus isolation of ITD, and sequencing quality is more difficult to describe. To initiate the accreditation of polio sequencing laboratories, sequencing standard operating procedure (SOP) was evaluated in several laboratories in 2010–2011. SOP for sequencing heterotypic and homotypic virus mixtures was circulated in July 2011. Serotype-specific VP1 sequencing method has been published (Kilpatrick et al, *J Virol Meth*, 2011, 174:128–130). Currently, US CDC is providing primers and has a plan to make a sequencing kit by the end of 2011. Still, GPLN’s sequencing needs should be estimated for phased-in implementation. A draft sequencing accreditation checklist was successfully piloted in three countries in 2011 and is being updated. In 2011, sequencing accreditation based on the draft checklist and site visit was initiated and will be fully implemented in 2012. A passing score for sequencing laboratory accreditation is >=90%. Some areas for improvement have been identified: dye removal process, sequence editing process, data management and documentation of sequence analysis and results. One of the challenges identified was the difficulty to review the edited trace files because laboratories use different software. US CDC is also considering sending two separate panels for rRT-PCR and sequencing. He also discussed the potential use of FTA cards for sending RNA samples and improving the accreditation process of polio sequencing laboratories. In the Western Pacific Region, real-time PCR proficiency test panel samples for polio ITD were distributed to five laboratories in the summer 2011, and all laboratories obtained a 100% score.

2.1.5 Country reports of national polio laboratories

(1) Hong Kong (China)

The Public Health Laboratory Centre (PHLC) was designated as NPL in Hong Kong (China) in 1992 and has performed ITD tests since 2002. PHLC is a centralized, biosafety level 2 virology laboratory that is used for the diagnosis of enterovirus infections and is responsible for AFP surveillance, enterovirus surveillance and serological surveys. The laboratory also receives AFP samples from Macau (China). Testing capacity is well established for direct detection, culture and molecular testing. A strong quality assurance programme, including SOP, staff training and participation in proficiency testing schemes, is in place. Since the introduction of IPV in 2007, the number of polio isolates tested for ITD has been very few. From 2009 to July 2011, around 81 AFP cases were investigated at PHLC. During this period, no poliovirus was isolated, and only a few NPEVs were identified from AFP surveillance. Only a few polioviruses and NPEVs were isolated from enterovirus surveillance. The results of a serological survey in 2010 showed high immunity (>99%) for polio1 and polio2 among all age groups and for polio3
among the following age groups: 1–10 years (95%), 11–20 years (93%), 21–30 years (83%) and >30 years (89%). Obtaining clinical samples from AFP cases and the low ITD workload are the challenges to maintain proficiency.

(2) Malaysia

The virology unit of the Institute for Medical Research (IMR) was designated as NPL in 1993 and has been fully accredited under WHO standards since 1998. In 2010, IMR was accredited as an ITD laboratory. Polio vaccine coverage is more than 90%, and no case due to wild poliovirus has been detected since 1993 in Malaysia. The laboratory is heavily involved in the investigation of HFMD, meningo-encephalitis and other infections due to enteroviruses. From 2009 to July 2011, IMR tested samples from 322 AFP cases and 4830 non-AFP cases. During this period, a total of 21 polioviruses (Sabin strains) were isolated from non-AFP cases, but no poliovirus has been isolated from AFP cases yet. The NPEV rate remains to be very low, but an increase in detection from 0.9% in 2009 to 4.1% in 2011 was noted. IMR is making efforts to address the low NPEV rate by obtaining a new batch of RD and L20B cell lines in July 2010 from VIDRL, Australia and will continue to perform cell sensitivity testing and report to the WHO Regional Office on a regular basis. Major areas of biosafety practice including personal protective equipment, emergency procedures, handling wastes and training are fully implemented in IMR. There was marked improvement on the timely submission of stool samples from the sentinel hospitals after close coordination of the laboratory staff with the hospital staff.

(3) Mongolia

The NPL in Mongolia received a total of 665 stool samples from 2009 to August 2011, consisting of 31 samples from AFP surveillance, 566 samples from a non-AFP survey (healthy children) and 68 samples for differential diagnosis. During this period, seven polioviruses (Sabin strains) were isolated from non-AFP cases and no poliovirus was isolated from AFP cases. Although only three (4.4%) NPEVs were isolated from AFP surveillance, stool samples from the healthy children survey were collected to supplement AFP surveillance. This helped NPL to achieve a high NPEV rate of 11.8%. A new batch of cell lines with absolute passage numbers were received from PHLC Hong Kong (China) in 2010, and a new batch of laboratory quality control (LQC) strains was prepared using the National Institute for Biological Standards and Control (NIBSC) strains. However, cell sensitivity test results in 2011 showed a decline in LQC titer. The head of the laboratory plays the lead role in staff training on biosafety issues since there is no designated biosafety officer at the institutional level. The laboratory must still work out how to involve silent provinces in the healthy children survey and how to prepare for wild poliovirus importation threats. Despite these challenges, the laboratory continues to perform satisfactorily and is fully accredited.

(4) New Zealand

The Ministry of Health is responsible for polio surveillance, laboratory and immunization activities. The Environmental Science and Research (ESR) clinical virology laboratory is the only WHO-accredited laboratory to conduct laboratory tests for all AFP cases in New Zealand. The workload of the laboratory is very low due to the small number of expected AFP cases (eight to nine cases per year). This laboratory is also involved in national enterovirus surveillance, which supplements AFP surveillance in New Zealand. From 2009 to June 2011, 20 stool samples from AFP cases were processed, and three (15%) NPEVs (two enterovirus 71; one enterovirus 74) were isolated. There were also 44 clinical samples received for enterovirus diagnosis during this period, and 13 (29.5%) NPEVs were detected. Cell sensitivity testing is performed regularly and is showing good results. The laboratory also performs conventional ITD PCR and VP1 sequencing. Due to the very low workload for AFP samples, laboratory staff need to spend more
time on maintaining polio technical capability. Establishing environmental surveillance is also being considered. For biosafety issues, the laboratory complies with AS/NZ PC2 structural and microbiological safety standards.

(5) Philippines

The Virology Department of the Research Institute for Tropical Medicine (RITM) was designated as NPL in 1991. RITM provides timely and accurate information on wild poliovirus importation and circulation of VDPV as well as virological evidence for certification. Laboratory performance is maintained according to WHO accreditation standards. Lack of human resources is a continuous issue with NPL. Currently, three staff members handle the laboratory’s entire polio workload, which is the highest among all network laboratories in the Western Pacific Region, except in China.

From 2009 to July 2011, a total of 2602 stool samples were received and tested from AFP surveillance, and 160 stool samples were processed from non-AFP cases. One stool sample was not processed due to inadequate quantity. The laboratory was also involved in the iVDPV study funded by WHO. A total of 29 polioviruses Sabin strains were isolated from all sources, of which, 26 polioviruses were from AFP cases and 3 polioviruses were from non-AFP cases. The NPEV rate remains low (<10%) despite efforts made to address the issue. Mycoplasma testing of RD and L20B cells using a PCR kit (e-Mycoplasm PCR Detection Kit) was initiated in March 2011 as recommended by WHO. Mycoplasma testing of cells showed evidence of contamination, and both corrective and preventive actions have been taken. NPL obtained a new batch of cell lines from VIDRL Australia in July 2011. Cell sensitivity is regularly performed, and the results are being submitted to the WHO Regional Office. There is a designated biosafety officer and all virology staff have undergone in-house biosafety training. Biosafety cabinets are certified annually by RITM Biomed engineers. All infectious wastes are autoclaved before disposal. The NPL staff have been involved in wild poliovirus containment activities and in the drafting of the Wild Poliovirus Importation Preparedness Plan of the Department of Health. Expanding enterovirus surveillance to include the healthy schoolchildren survey and HFMD/meningitis are planned in 2012 to supplement the AFP surveillance.

(6) Republic of Korea

The National Vaccine Programme introduced OPV in the 1960s, and the last wild poliomyelitis case was reported in 1983. IPV was introduced in 2005, and the estimated immunization rate is over 95%. No poliovirus has been detected since 2006. The laboratory supports the AFP and enterovirus surveillance system in the Republic of Korea. From 2009 to August 2011, the laboratory processed 236 stool samples from 108 AFP cases. A research project to facilitate collection of stool samples from AFP cases and data sharing is ongoing to improve the AFP surveillance in the Republic of Korea. More than 5000 samples were received and tested from the Enterovirus Surveillance Network in 2009 and 2010. All polioviruses detected from the enterovirus surveillance were Sabin-like. The NPEV detection rate by PCR was 27% during this period. Cell sensitivity data showed satisfactory results for both RD and L20B cell lines. A major outbreak of EV71 with neurological complications occurred in 2009 and 2010, and three patients died. For the detection of enteroviruses, including EV71, real-time PCR and conventional PCR are conducted. The detection rate of EV71 was very high at 51.8%.

(7) Singapore

An AFP surveillance system was established by the Ministry of Health in 1995, and the Singapore General Hospital laboratory has been the WHO-accredited NPL since 1998. This laboratory receives samples from an "enhanced" AFP surveillance system as well as
environmental samples. The "enhanced" surveillance system requires public hospitals to notify the Ministry of Health of all patients with diseases that could lead to AFP whether or not AFP is present. From 2009 to June 2011, a total of 1338 samples were processed for enterovirus culture, including 45 stool samples from AFP cases, 1182 samples of various types from non-AFP cases and 111 raw water samples. Three polioviruses were isolated in 2010. The NPEV isolation rate from stool samples was >10% and 7%-15% from other sources. Cell sensitivity testing is performed regularly and the results have been very satisfactory. The laboratory has established capacity for sequencing and has requested standardized protocol as well as primers and reagents from WHO for polio VP1 sequencing.

(8) Viet Nam (Ha Noi)

The NPL in the National Institute of Hygiene and Epidemiology (NIHE) is responsible for the northern provinces of Viet Nam. The laboratory received a total of 804 stool samples from AFP surveillance from 2009 to July 2011. Three polioviruses were isolated in 2009 and 2010. No poliovirus has been isolated as of September 2011. Fifty-eight (7%) NPEVs were isolated during this period. Laboratory testing of samples from enterovirus surveillance is also conducted, and 350 samples were received from HFMD cases. From HFMD samples, 180 NPEVs (51.4%) were detected. Among NPEVs, 65 (36%) were EV71, 52 (31%) were coxsackievirus A viruses, 2 (1%) were coxsackievirus B viruses and 53 are pending results. Since cell sensitivity testing results are not stable, a new batch of RD and L20B cell lines will be obtained. Training of new staff is needed, and WHO's support in the provision of some equipment was requested.

(9) Viet Nam (Ho Chi Minh City)

The Laboratory of Enteroviruses in the Pasteur Institute in Ho Chi Minh City is responsible for testing samples from AFP surveillance in southern Viet Nam. The new testing algorithm was implemented in 14 May 2010. From 2009 to 20 August 2011, stool samples from 716 AFP cases were received and processed, and 8 polioviruses and 88 (12.3%) NPEVs were isolated. During this period, the laboratory also tested 1353 various clinical samples from children with HFMD or central nervous system infections by PCR. Among these samples, 982 (72.6%) NPEVs were detected, and out of these NPEVs, 524 (53.4%) were EV71 and 458 (46.6%) were other NPEVs. Virus isolation was done on the 982 positive samples and no poliovirus was isolated. There is also close coordination between the laboratory and EPI staff. A WHO biosafety manual was translated into Vietnamese, and all laboratory staff must attend biosafety training before entering the laboratory. A new batch of RD and L20B cell lines and standardized SOP for the ITD function to introduce real-time PCR for polio ITD were requested.

2.1.6 Detection of poliovirus from non-AFP cases

(1) Environmental surveillance of poliovirus and non-polio enteroviruses in China

Dr Yong Zhang presented on the progress of environmental surveillance in China, which aims to monitor the importation of wild poliovirus in border provinces in western China, to enhance VDPV surveillance in high-risk areas with low vaccine coverage or areas in central provinces where VPDVs were previously detected, and to assess the effect of SIAs before and after OPV campaigns in eastern provinces. Environmental surveillance was established to supplement AFP surveillance activities, to predict enterovirus-associated diseases and to detect emerging or re-emerging pathogens in China. Three training workshops on environmental surveillance have been conducted from 2007 to 2010. Results of environmental surveillance in Shandong, Guangdong, Xinjiang and Tibet were presented. In Shandong province, a total of 50 sewage samples were collected during the period 2008–2010. Of these, 37 samples were enterovirus positive (74%), yielding 19 polioviruses and 186 NPEVs. All polio isolates were
Sabin strains (three type 1, nine type 2 and seven type 3). No VDPV was isolated. Out of 186 NPEVs, 171 isolates were identified into 15 serotypes. The analysis showed that there was an epidemic of echovirus 6 in Jinan City. In Guangdong, samplings were taken from sewage treatment plants and rivers from 2008 to 2010. From sewage samples, 894 NPEVs and 120 polioviruses (26 P1, 58 P2 and 36 P3) were detected, and from river samples, 60 enterovirus strains and 47 polioviruses (4 P1, 23 P2 and 10 P3) were identified. Since 2010, the environmental surveillance project in China has been expanded to 10 provinces. Virus isolation results from environmental and clinical sources could provide complementary data to trace prevalent and silent circulating enteroviruses in the human population.

(2) Environmental surveillance for poliovirus in Australia

In Australia, IPV has been used since November 2005 and environmental surveillance was initiated in 2010. Dr Bruce Thorley described the testing protocol of environmental samples, which adopted the WHO protocol established by the polio laboratory in Finland. Environmental surveillance sites were selected in collaboration with local public health units and local wastewater utilities from rural cities. Three sampling sites in Armidale, Newcastle Shortland and Byron Bay were chosen, and two methods were used for the study: (1) direct detection of human enterovirus (HEV) in concentrate by in-house pan-enterovirus semi-nested RT-PCR (panEV snRT-PCR), and (2) human enterovirus identification from isolates (cell culture) followed by CODEHOP RT-PCR. No poliovirus was detected.

2.1.7 Experience of polio laboratory network for the laboratory diagnosis of hand, foot and mouth disease and other enteroviruses

(1) Hand, foot and mouth disease surveillance in China

Dr Yong Zhang presented on HFMD surveillance, molecular epidemiology of enterovirus 71 and coxsackievirus A16, and seroepidemiology of HFMD in mainland China. HFMD associated with CA16 was first reported in 1957 in Canada. In 1959, a name for the disease—hand, foot and mouth disease—was designated. Since then, several large outbreaks of HFMD with high incidence of neurologic infection and fatality have been reported in Asia and the Pacific, e.g. Malaysia in 1997, Australia in 1999, and Taiwan (China) in 1998. In China, a large-scale outbreak of HFMD associated with EV71 occurred in Linyi city, Shandong province, with 1149 mild and 3 fatal cases in 2007. A nationwide epidemic of HFMD occurred in 2008, starting as an outbreak of unknown viral infection in Fuyang city of Anhui province in spring 2008, which was later identified as an EV71 outbreak. HFMD was included into the National Notifiable Disease Report System (NNDRS) as the 38th legally notifiable disease on 2 May 2008. The epidemics were widespread in 2009 and 2010. A total of 1 771 548 HFMD cases were reported in 2010, and among those cases, 27 913 were severe cases that resulted in 905 deaths.

Based on the model of the WHO polio and measles laboratory networks, a three-level HFMD laboratory network was established in 2008. Detection methods used for HFMD diagnosis from clinical specimens were RT-PCR and rRT-PCR for pan-enterovirus, EV71 and CA16 as well as virus isolation and sequencing. In 2008, the laboratory results showed that EV71 accounted for 81.59% of severe HFMD cases and 96.43% of deaths. From 2009 to 2011, EV71 accounted for >80% of laboratory-confirmed severe cases and >92% of laboratory-confirmed fatal cases. Genotyping of EV71 based on VP1 gene was done from 1987 to 2010; the results showed that the first EV71 isolate belonged to the C2 genotype, followed by C3 in 1997 and then C4 strains predominated from 1998. From 1998 to 2009, subgenotype C4 evolved due to its circulation for 11 years in mainland China. The phylogenetic analysis of the complete genome of EV71 detected from 1998 to 2009 showed that there might be recombination events between EV71 and CA16 strains that were persistently circulated in China for at least 13 years.
Seroepidemiological studies of healthy populations from six provinces in 2005 confirmed that EV71 and CA16 had widely circulated in China before the large-scale outbreaks occurred in 2008.

(2) Australian national enterovirus surveillance, 2009–2011

Dr Jason Roberts reported the outcomes of enhanced surveillance in Australia, which was introduced in 2009 to supplement existing clinical/laboratory surveillance and possibly address surveillance failure (specifically stool collection rates). Enhanced surveillance includes environmental surveillance, enterovirus surveillance and enhanced/rapid high throughput polio/NPEV detection and identification. For the detection of enteroviruses, PanEV snRT-PCR is used but some cross reactivity with human rhinovirus was observed. Parallel testing by PCR and culture was done on 230 samples in 2010–2011, and the results showed 29 (12.6%) were PCR-positive but culture-negative. From 2007 to August 2011, a total of 1156 HEV samples and typing results were received at VIDRL. In October 2009, Sabin poliovirus of unknown origin was detected though there was a cessation of OPV use. It was also noted that NPEV identification increased from October 2009. In Australia, EV71 subgenotype B5 strains, which were highly homologous to EV71 detected in Singapore in 2008 and in Taiwan (China) in 2007 and 2009), were detected during the period 2009–2011. He also described briefly the molecular modelling project on "All-atom model of the poliovirus RNA and capsid", which uses parallel-computing software that simulates biomolecules and visualizes the results of those simulations.

(3) NIID polio laboratory activity: NPEV surveillance and laboratory diagnosis

Dr Hiroyuki Shimizu reported on NPEV surveillance and laboratory diagnosis in Japan. He described the clinical diagnosis of EV71 infection, which can be asymptomatic and has various symptoms such as HFMD, aseptic meningitis and severe neurological complications. EV71 is considered to be one of the most neurotropic enteroviruses after poliovirus is eradicated. However, no vaccine or antiviral agent is available for EV71 at present, partly due to the lack of appropriate animal models. He also described the prevalence of EV71 subgenogroups in the Asia Pacific region from 2005 to 2010. Several severe HFMD outbreaks occurred as early as the 1970s in Europe and the 1990s in Malaysia and Taiwan (China). From 2000 to 2010, severe HFMD outbreaks caused by EV71 occurred in China, Viet Nam and other Asian countries, and subgenotypes C5, C4, C1, C2 and B5 strains were confirmed. New diagnostic approaches for enterovirus diagnosis are being used such as real-time PCR, real-time loop-mediated isothermal amplification (RT-LAMP) and immunochromatography for bedside diagnosis. The RT-LAMP method is mainly for polioviruses and HEV-C (high sensitivity), with isothermal one-tube reaction (low contamination risk), and the reagents are commercially available except primers. The RT-LAMP method has a low sensitivity for detection of HEV-A (EV71) and HEV-B and further identification is needed. In 2011, Japan experienced the highest level of HFMD cases caused by coxsackievirus A6, showing extended skin blisters on arms, legs and buttocks as well as hands and feet. The data also showed prevalence of different NPEV serotypes (CA16, CA10, CA6, CA4, CA5 and EV71) among HFMD cases in Japan from 2007 to 2011.
2.1.8 Data management and communication

(1) Update on standardization of AFP laboratory database

Dr Fem Julia Paladin presented updates on the standardization of AFP laboratory data management. Developing a standardized polio laboratory databases programme in the Western Pacific Region was recommended at the second VPD laboratory networks meeting to support new diagnostic approaches in GPLN and the expansion of scope of regional polio laboratory network activities. A new standardized polio laboratory database was drafted to include core variables such as epidemiology and case information, virus isolation with options for poliovirus typing, ITD and sequencing, and to capture the new virus isolation algorithm. Other core variables were also proposed to capture workload and indicators from other sources, e.g. enterovirus surveillance, special studies (iVDPV), HFMD, diagnostic testing, environmental surveillance and healthy children surveys. Investigation of non-AFP cases should also be reported as aggregate data by month and year and shared with WHO as often as AFP cases. Data checking will minimize data entry errors and missing information. A new database could generate feed-forward and feedback reports automatically to ensure consistent data and also to promote efficiency, data export features for on-site data analysis of workload, performance indicators as well as data for publications. Few laboratories have provided feedback on the proposed database variables distributed during the second VPD meeting. Until the new database is finalized and available, network laboratories can report using the current reporting format at least monthly, no later than the 10th of the following month.

(2) Demonstration: Draft AFP database application

Mr Benjamin Bayutas presented the new polio laboratory database. He stated that the challenge in the current practice of reporting is the submission of Excel spreadsheets, with varying formats, e.g. Epi Info format (*.REC) and Access database format (*.MDB), by network laboratories. The use of a new standard reporting format will guarantee the reporting of all core variables, automatic data checks, reliable data transfer, automatic report generation, utilities for feed-forward, backup and restore, and improved data archiving. The new database was also demonstrated. An updated draft version will be released later in 2011 and will be rolled out/implemented in 2012.

2.2 Biosafety training

2.2.1 Introduction to biosafety training

WHO Headquarters developed biosafety training materials in 2009 in collaboration with laboratories in Finland and Zambia. The training materials were developed for trainers and not for hands-on training. The training package was designed to be used by all staff in a poliovirus laboratory including support staff. The six modules that have been developed aim to share important messages and good practices, to stimulate discussion and promote class participation, to encourage staff to share opinions and experiences on safely working with poliovirus in the laboratory, and lastly, to help identify areas for improvement where work can be performed more safely and minimize the risk of release of poliovirus to the environment.

Dr Youngmee Jee presented the six modules of the biosafety training, namely: emergency procedures; laboratory infrastructure; equipment; personal protective equipment (PPE); disinfection, autoclaving and waste management; and training. Each module has a leaflet (with key messages, questions and “spot the mistakes”), a presenter’s note with suggested schedule and instructions for trainers, a prompt card, and a video (three scenes), showing deliberate mistakes
and key messages. Each set of training materials also includes training guidelines, feedback form, post-training questionnaire and certificate.

Before conducting the biosafety training, a suggested schedule for each module was presented, participants from network laboratories were divided into groups, and facilitators/trainers and rapporteurs/presenters were identified for each group.

The participants were divided into three groups as follows:

Group 1: Australia (2), Fiji (2), Malaysia (3), New Zealand (3), Papua New Guinea (2)

Group 2: Cambodia (2), Lao People’s Democratic Republic (2), Mongolia (3), Philippines (5), Viet Nam (3)

Group 3: China (7), Hong Kong (China) (3), Macao (China) (1), Japan (4), Republic of Korea (2), Singapore (2)

2.2.2 Training schedule and modules

Training schedule for each module:

<table>
<thead>
<tr>
<th>Time</th>
<th>Proposed schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>Welcome and distribution of leaflet for module</td>
</tr>
<tr>
<td>10 min</td>
<td>Answer questions in leaflet</td>
</tr>
<tr>
<td>5 min</td>
<td>Watch video segment 1</td>
</tr>
<tr>
<td>10 min</td>
<td>Discussion for video segment</td>
</tr>
<tr>
<td>5 min</td>
<td>Watch video segment 2</td>
</tr>
<tr>
<td>10 min</td>
<td>Discussion for video segment</td>
</tr>
<tr>
<td>5 min</td>
<td>Watch video segment 3</td>
</tr>
<tr>
<td>10 min</td>
<td>Discussion for video segment</td>
</tr>
<tr>
<td>10 min</td>
<td>Q&amp;A - Discussion of module and how we do it</td>
</tr>
<tr>
<td>10 min</td>
<td>Participants to review their answers to the questions in the leaflet</td>
</tr>
<tr>
<td>10 min</td>
<td>Presenter to discuss answers to the questions in the leaflet</td>
</tr>
<tr>
<td>5 min</td>
<td>Participants to fill in post-training questionnaire and issue prompt cards</td>
</tr>
</tbody>
</table>

*(For this training only after the last module)*

Six modules of the training package:

1. Disinfection, autoclaving and waste management

This module aims to send important safety messages in the management of laboratory wastes to reduce the risk of poliovirus transmission to the laboratory workers, the general public and the environment. Decontamination in the laboratory needs to be planned and carried out with
great care. It involves disinfection of work surfaces and equipment, as well as safe treatment and disposal of laboratory wastes.

(2) Equipment

The aim of this module is to share essential messages about the safe and effective operation of frequently used equipment in the poliovirus laboratory. The module emphasizes considerations when purchasing equipment, the need for preventive maintenance and the safe removal of equipment from the poliovirus laboratory for servicing and disposal. The use of a backup generator and uninterruptible power supply (UPS) is also discussed.

(3) Laboratory infrastructure

The purpose of this module is to share important safety messages about the design of the poliovirus laboratory. This includes working areas and conditions that are known to pose safety problems.

(4) Personal protective equipment (PPE)

This module intends to share vital safety messages about the use of PPE and other safety equipment in the laboratory. The types of PPE that should be used when handling liquid nitrogen and safety precautions to observe when preparing disinfectant, and other toxic substances are discussed. Good laboratory practices when working in a biological safety cabinet are also highlighted.

(5) Emergency procedures

The aim of this module is to share crucial messages about emergency situations and how the laboratory should respond. Emphasis is placed on how a laboratory can prepare for a fire emergency and how a spill of poliovirus should be handled. A fire hazard inspection is also carried out to highlight some common issues encountered in a laboratory.

(6) Training

This module aims to explain the importance of training for all laboratory personnel, including the significance of defining training needs for different groups and the need for close supervision of new staff. If personnel are well trained and competent, the risk of incidents or accidents in the laboratory will be significantly reduced. This module proposes that a training plan should be revised after a number of accidents and incidents, and the biosafety officer struggles to create an effective biosafety training programme.

Evaluation of the training:

The training was interactive, relevant and interesting, and the aims of biosafety training were met. After the workshop, network laboratories were provided with the training materials and were requested to organize similar training in their own laboratory and to provide feedback to the WHO Regional Office within six months.
2.3 Measles and Rubella Laboratory Network

Opening session

Dr Youngmee Jee, regional EPI laboratory coordinator, welcomed all participants and advisers. The Director of Programme Management, Dr Hans Troedsson, opened the session and welcomed participants from countries and areas and advisers from partner organizations. He acknowledged the contribution of the laboratory network during the last few years and emphasized the critical role of the Measles and Rubella Laboratory Network to achieve the regional measles elimination goal by 2012.

2.3.1 Overview of global and regional measles elimination and rubella control initiatives

(1) Global and regional measles and rubella updates

Dr David Sniadack made a presentation on global and regional updates on measles and rubella. The Region had already achieved 93% reduction of measles morbidity by 2011 compared to 2000. Between 2010 and 2012, measles SIAs were conducted and planned in 13 countries in the Region. Measles incidence in the Region dropped from 82 per million in 2008 to 27 per million in 2010, and 18 per million in 2011. He presented measles surveillance performance indicators including reporting rates, adequacy of investigation, percentage of laboratory confirmation, percentage of virus detection and percentage of clinically confirmed cases. Reporting rates should be >2 discarded measles cases per 100 000 population per year and >1 in at least 80% of subnational administrative units. According to the countries’ monthly reports, the proportion of laboratory-confirmed measles cases is increasing in most countries. While China made good progress in reducing measles incidence after a nationwide SIA in September 2010, measles incidence in 2011 varied greatly between the western and eastern provinces. Western provinces such as Xinjiang and Tibet had higher incidence of measles in 2011. The Philippines also experienced outbreaks of measles in 2010 and 2011, and Cambodia had increased incidence of measles in 2010 and 2011. Malaysia also experienced a measles outbreak in 2011, most notably in Sabah province. Genotyping of measles virus is becoming more important for differentiation of imported cases from endemic cases. Australia detected many imported cases from France, India, Indonesia, Malaysia, the Philippines, Singapore and Thailand with genotype information available.

The Region is also accelerating rubella control and congenital rubella syndrome (CRS) prevention. While reported rubella cases are declining from 2008 levels, severe CRS cases are still reported as the result of rubella outbreaks among adults. An updated WHO position paper on rubella recommends that the countries should use rubella-containing vaccine for two-dose measles vaccinations. The preferred approach to introduce rubella vaccines would be conducting MR or MMR vaccination in a wide-age-range campaign followed by introduction of MR or MMR vaccine in the routine immunization programme to achieve/maintain 80% coverage through routine or regular SIAs. Regional plans for eliminating measles by 2012, including conducting quality SIAs and improving routine coverage in countries, strengthening epidemiologic and laboratory surveillance and communication, accrediting all RRLs and NMLs as well as establishing regional and national verification committee for measles elimination, were presented. To strengthen surveillance, improving sensitivity at the subnational level, better contact-tracing to establish epidemiolocal linkages and increased collection of throat swab samples to identify measles virus should be emphasized. Countries that have eliminated or are near measles elimination may begin the verification process.
(2) Global update on measles and rubella laboratory network

Mr David Featherstone presented an update of the global measles and rubella laboratory network. The network has grown from 80 laboratories in 2001 to 690 laboratories including 362 subnational laboratories in China in 2011. In 2011, 183 out of 193 countries were being served by proficient laboratories. The network processed 200 000 to 300 000 samples for measles and rubella IgM testing per year during the period 2007–2010, with a high level of proficiency. Including samples processed in China’s network laboratories, about half of the samples were processed in Western Pacific Region network laboratories with reporting timeliness of >90%. All network laboratories that participated in WHO proficiency testing (PT) for measles and rubella IgM ELISA passed with scores of >90%. Detailed analysis of OD values of PT results received from the network laboratories that used Siemens, Haitai, Vierion Serion and Kerunda kits was also presented. Developing an external quality assurance programme for PCR detection, real-time PCR, new sequencing primers, standardized measles serosurvey and rapid point-of-care assays were presented. The progress made for measles elimination and rubella control was published in the *Journal of Infectious Diseases* in 2011, which included 10 laboratory-related papers. At a measles and rubella research meeting in May 2011, the following laboratory components were discussed: vaccine immunogenicity and efficacy, monitoring changing maternal immunity to measles, waning immunity, new vaccines and novel or alternative routes of vaccination, diagnostics, molecular epidemiology, serological assays and point-of-care diagnostics and distinction of vaccine and wild type induced antibodies and laboratory quality control or molecular epidemiology. Training workshops were held in each region to strengthen laboratory capacities. From 2010 to July 2011, 171 participants from 80 laboratories in 41 countries were trained at WHO-organized workshops. In the Western Pacific Region, 87 participants from 41 laboratories in 14 countries were trained. During a measles outbreak in South Africa in 2010–2011, the laboratory processed a huge number of samples to meet increased surveillance needs. Implication for the laboratory network in achieving measles elimination was presented. More than 80% of laboratory-confirmed measles outbreaks should have adequate samples for virus characterization in a WHO-accredited laboratory. However, data reporting and reconciliation between laboratory and surveillance data can be improved. Changes were made for the accreditation checklist to be used to review subnational laboratories in China. Identification of resources for capacity-building and extra testing remains as a challenge.

(3) Updates of regional measles and rubella laboratory networks

Dr Youngmee Jee provided an update on the measles and rubella laboratory network in the Western Pacific Region. As of August 2011, 47 out of 48 fully functional network laboratories including 31 provincial laboratories in China have been accredited. In 2010, about 60 000 samples were tested for measles (42 976 from China and 16 673 from other countries) and 4563 samples were positive for measles IgM (27%). Among 14 358 samples tested for rubella IgM, 4040 samples were positive for rubella IgM (28%). More network laboratories have been performing virus isolation and genotyping after the hands-on training in 2009 and 2010 in Hong Kong (China) RRL. The measles laboratories in China, Japan, Malaysia, Mongolia, New Zealand, the Philippines, the Republic of Korea, Singapore and Viet Nam are performing or establishing PCR and/or genotyping capacities. Measles genotypes identified in 2010 were H1, D4, D8, D9, G3 and B3. Endemic genotypes in this Region include H1 (China, Lao People’s Democratic Republic, Viet Nam) and D9 (Cambodia and Philippines). G3 strains were detected in Malaysia and Singapore in 2010–2011, and in Japan in 2011, but have not been detected in the Philippines since 2009. D9 strains have been widely detected in the Region: Australia, China, Hong Kong (China), Japan, Malaysia, the Republic of Korea and Singapore. Mixed genotypes related to importation were reported from Australia, Hong Kong (China) and Japan in 2010–2011. D5 strains have been detected in Japan since May 2010, and D4, D8, D9 and G3 strains have been detected in 2011. Except China, which processed around 40 000 samples per year in 2009–2010,
Cambodia, Malaysia, the Philippines and Viet Nam processed the largest number of samples during 2009–2011. Rubella-positive cases have been increasingly detected in the Region, especially in Cambodia and Viet Nam in 2011. In 2010, all 49 laboratories reported the correct results for the WHO proficiency test for measles and rubella IgM, but one laboratory did not report OD values of each sample. Of the 48 laboratories, all of them scored 100% for measles IgM, and for rubella, 39 labs scored 100%, seven labs scored 95% and one lab scored 90%. In 2011, 20 additional sets of PT samples will be sent to Japan (17), Viet Nam (2) and Malaysia (1). Confirmatory testing of a proportion of samples from national laboratories was performed in RRLs in Hong Kong (China) and Australia. The concordance rates improved in 2010–2011, and concordance rates of >90% were observed for measles and rubella testing for samples from most laboratories. Completeness and timeliness of laboratory reporting gradually improved from 2008 to 2011, reaching 86% and 78%, respectively. Implementation of the recommendations from the last meeting in 2010 was reviewed. Mumps outbreaks also occurred in Mongolia and the Lao People’s Democratic Republic, and genotypes were confirmed by Hong Kong (China) RRL as F and G1, respectively. Hong Kong (China) RRL plays a major role by conducting most of the confirmatory testing and genotyping in the Region. Remaining challenges include quality assurance of laboratory confirmation of measles outside WHO network laboratories in commercial laboratories in Japan, Singapore and other public health laboratories in Malaysia and Viet Nam. The WHO Regional Office is planning to include those laboratories in the network by sending WHO PT samples and conducting on-site review for WHO accreditation. Since VIDRL is not performing rubella genotyping, samples for rubella genotyping will be sent to Hong Kong (China) until national laboratories can perform rubella genotyping with proficiency. Planned activities in 2011 and 2012 include further strengthening of strain surveillance for measles and rubella, use of dried blood spot in Cambodia, Fiji, Papua New Guinea and the Philippines, full implementation of newly improved database and conduct of a follow-up, hands-on training in Hong Kong (China) in 2012.

2.3.2 Quality assurance

(1) Measles and rubella IgM proficiency testing in the Western Pacific Region

Ms Jennie Leydon from VIDRL, Australia presented the purpose of measles and rubella IgM proficiency testing, the procedures of preparing WHO measles and rubella IgM proficiency testing samples, and the analysis of results of 00905 PT samples, which were used for 218 network laboratories, and 01005 PT, which were used for 223 network laboratories. In the Western Pacific Region, 49 laboratories including 31 provincial laboratories in China participated and all passed with scores of >90%. Network laboratories in this Region used Siemens, Haitai, Virion Serion, Trinity Biotech and Denka Seiken IgM kits for measles IgM ELISA, and Siemens, Haitai, Kerunda, Virion Serion, Wamploe, Biorad and Denka Seiken kits for rubella IgM ELISA to test PT samples. One laboratory in the Western Pacific Region used an expired rubella kit and one country did not indicate the expiry dates of measles and rubella IgM kits used for WHO PT. Out of 50 laboratories that received 00905 PT samples, 48 labs scored 100% for both measles and rubella, two labs scored 95% for measles and two labs scored 95% for rubella. Laboratories with enough positive samples were requested to contribute positive samples for preparing PT.

(2) Confirmatory testing in Hong Kong (China)

Dr Janice Lo from Hong Kong (China) RRL presented objectives and principles of quality control and variables affecting the quality of testing such as competence of personnel, quality of kits and equipment, conditions of specimens, controls used with the runs, interpretation of data, transcription of results and reporting results. Two types of controls—internal and external—are used for quality control. Internal controls are supplied with a commercial kit and are lot
dependent, while external controls are not a part of commercial kits and in-house diagnostic samples or samples from another laboratory can be used to monitor lot-to-lot variation to verify each test run. In-house control samples will provide assurance that the assay is performed appropriately with consistent results, monitoring batch-to-batch variation of reagents and day-to-day or inter-assay variation. In-house positive control samples should be low positive in the range of cut off and positive control to be able to identify any minor changes in assay performance which may not be easily recognized by reviewing negative and positive kit controls. Accuracy in determining true infection status varies with the prevalence of infection in the population. The higher the prevalence, the greater the probability that a person testing positive is truly positive. Disease prevalence and positive predictive value of the testing were also explained. When disease prevalence is low, false positive rates would be high and positive predictive values of the testing would be low, but when disease prevalence is high, false positive rates would be low and positive predictive values of the testing would be high. Confirmatory testing results of samples from nine national laboratories were presented from 2009 to 2011. The concordance rates varied from 92.3% to 100% for measles and from 86.7% to 100% for rubella IgM.

(3) Update on quality assurance in the measles laboratory network in China

Mr Mao Naiying from China CDC, WHO RRL presented quality assurance measures such as proficiency testing, confirmatory testing, WHO accreditation review and use of in-house control samples, which are used in China’s measles network laboratories. All provincial laboratories received WHO PT samples from VIDRL and scored 100% on the proficiency test. For the 2010 PT, 20 laboratories used Haitai measles IgM kit, 10 used Verion Serion and 1 used Trinity, while 18 laboratories used Haitai rubella IgM kit, 10 used Kerunda, 2 used Verion Serion and 1 used Trinity. China CDC performed detailed analysis of OD values received from the laboratories, and significant variance was observed for locally produced kits when compared to Siemens kit. For confirmatory testing, each provincial laboratory sent 50 samples consisting of 20 measles-positive, 10 rubella-positive and 20 negative samples. Concordance rates of measles IgM and rubella IgM were >95% and >90%, respectively. It was highlighted that one of the key methods for quality assurance is to use in-house controls for the assays and to monitor the optical density values of in-house controls in addition to the kit positive and negative controls. Concordance rates were 100% for all laboratories in 2010, while eight laboratories did not obtain 100% in 2009. It was noted that only strong positive samples were sent to China CDC for confirmatory testing and that some provinces could not send 20 measles-positive cases due to decreasing measles incidence in recent years. The Tibet CDC laboratory was reviewed in 2010 for the first time and passed WHO accreditation. The use of in-house control samples for ELISA was introduced in 2006 to monitor batch-to-batch variations. China CDC provided SOP for preparing in-house control samples. To ensure the quality of testing in prefectural laboratories, provincial laboratories prepare in-house control samples and distribute them to the prefectural laboratories. However, some provincial laboratories did not properly implement the use of in-house control samples for ELISA testing. China CDC organizes annual training for 10–20 provincial laboratories to provide training opportunities for ELISA, virus culture and real-time PCR.

(4) Developing quality assurance programme for molecular techniques

Dr Paul Rota from US CDC presented the importance of quality control for molecular tests as most regions are expanding virological surveillance for measles and rubella. He presented diagnostic RT-PCR and genotyping kit with validated primer and probes, positive controls and FTA practice panels to support quality control of molecular testing among network laboratories. He also presented ways to avoid contamination when working with RNA. One should use dedicated equipment, rooms and hoods for all pre-amplification procedures, separate master mix and RNA extraction, post-amplification analysis and processing using dedicated equipment.
Equipment and lab coats should not be shared between pre-PCR and post-PCR rooms. Filtered tips should be used for all pre-PCR procedures and for setting up RT-PCR reactions. Gloves should be changed often, and repeated freezing and thawing of samples should be prevented to prevent RNA degradation. Control RNA used in measles genotyping PCR and real-time PCR is the synthetic RNA with an insert of 220 nucleotides (854 bp) to reduce the risk of misinterpretation due to cross-contamination.

Two synthetic RNA positive control constructs are used for rubella genotyping. One control contains an insertion of 30 nucleotides and is used for diagnostic RT-PCR and fragment 1 genotyping RT-PCR. The other control has a deletion of 84 nucleotides and used for fragment 2 genotyping RT-PCR.

For the quality control of genotyping RT-PCR, both positive and negative controls should be used in all assays. It is good to use US CDC positive controls since those controls have extra insertions or deletions. It is also important to maintain proper workflow from clean to dirty areas. Molecular practice panels prepared with Whatman FTA cards are chemically treated to lyse cells and denature proteins on contact and samples are not infectious. Advantages of using Whatman FTA cards are: (1) nucleic acids are protected from microbial and fungal attack, (2) samples can be stored and shipped at room temperature, (3) reduced shipping costs, (4) they can be used for shipping virus isolates, and (5) it is an efficient and low-cost way to provide positive controls for standard measles and rubella RT-PCR reactions. However, these cannot be used for transporting serum samples.

2.3.3 Investigation of measles outbreaks and the role of the laboratory

(1) The Philippines experience

Dr Amado Tandoc from national measles laboratory (NML), RITM presented the epidemiology of a measles outbreak between 2009 and 2011 in the Philippines and the role of the laboratory in measles surveillance. The NML plays a major role in monitoring and verifying virus transmission by identifying measles virus strains and in monitoring the susceptibility profile of the population. In 2010 and 2011 (as of August), 2527 and 3524 cases were confirmed as measles IgM positive, respectively. During April–May 2011, a door-to-door immunization campaign targeted 18.5 million children aged 9–95 months using measles rubella vaccine. In 2010, 123 samples were received for virus isolation, and 35 measles and 5 rubella virus strains were identified. During January–April 2011, 100 virus isolation samples were received, and 19 measles strains were identified. In 2010 and 2011, measles genotype D9 strains were detected from Regions 3, 4A, 9, 11 and 12 and the National Capital Region, and rubella genotype 1j and 2B strains were detected from the National Capital Region and 1j strains from the Cordillera Autonomous Region. He also presented the plan to strengthen rubella and CRS surveillance and molecular techniques.

(2) Viet Nam experience

Dr Trieu Thi Thanh Van from NIHE presented the epidemiology of measles from 2009 to 2011 in Viet Nam. A measles outbreak that peaked in 2009 has since ended. The laboratory provided confirmation of outbreaks through measles IgM detection and identification of measles and rubella virus strains and genotypes during the outbreaks in collaboration with Hong Kong (China) RRL and WHO.
2.3.4 Enhancing molecular surveillance

(1) Molecular surveillance of measles virus in Japan

Dr Katsuhiro Komase presented two different systems of confirming measles cases in Japan: (1) most measles ELISA testing is done in commercial laboratories, and (2) prefectural institutes of public health are conducting RT-PCR for confirming measles cases in Japan. Since Japan introduced the measles elimination plan and case-based reporting in 2008, the number of measles cases dropped from 4202 in 2008, to 428 in 2009 and to 329 in 2010. Also, the proportion of case confirmation by PCR increased and about half of measles cases were confirmed by PCR in 2011. Measles genotype D5 strains, which used to be endemic in Japan, have not been detected since 2010. In 2011, 44 D9 and 54 D4 strains were detected in Japan. In addition, a few D8, H1 and G3 strains were also detected in 2010-2011. Most cases were considered to be imported or import-related cases although epidemiological information was not available for all cases.

(2) Update of measles virus surveillance in China

Dr Yan Zhang presented updates on measles surveillance in China and testing algorithm of virus surveillance samples in China CDC and provincial laboratories. Most provincial laboratories can perform virus isolation and RT-PCR, and some laboratories can also perform sequencing or send out samples to commercial laboratories for sequencing. China CDC receives isolates from provincial laboratories for sequencing, provides feedback to provincial laboratories and also sends genotyping results to WHO on a monthly basis. From 1993 to 2011, 2273 measles strains were genotyped, and H1 strains were detected predominantly from 2255 cases. From 2009 to 2011, D4, D9, D11 strains were detected from imported cases in five provinces. Measles genotype information is available from 30 provinces except Tibet. She presented information on a measles outbreak caused by new D11 measles strains in Menglian county of Yunnan Province, which has a border with Myanmar.

(3) Importation of measles viruses into Australia, January 2010–June 2011

Dr Doris Chibo from VIDRL, Australia presented strategies of PCR testing for measles and imported measles cases in Australia between January 2010 and June 2011. VIDRL provides free service in performing PCR on sample with an IgM positive result for public health request including genotyping confirmation for interstate laboratories, which send clinical samples, virus or cDNA. For diagnostic purposes, all samples are initially tested using measles real-time PCR. For genotyping purposes, all real-time positive samples are tested by conventional PCR followed by sequencing. Virus isolation is conducted only when a novel subtype has been identified and reference strain is required. From January to June 2011, 324 samples were tested by real-time PCR. Of those tested, 103 were positive and 72 including six D9 strains from New Zealand were genotyped. Age distribution of genotyped measles cases shows that people over 40 years old who missed vaccination are also infected. Some clustering of cases under 35 years old suggests that the measles vaccination programme is still missing some people. A diverse range of importations of measles viruses occurred, and under or unvaccinated people are potentially fuelling transmissions and impacting herd immunity.

(4) Molecular epidemiology of measles virus - experience of the Republic of Korea

Dr Kisoon Kim from Korea CDC presented a nosocomial outbreak in 2007, a modified measles outbreak in one middle school in 2010 and import-related cases caused by D9 strains in 2011. H1 strains were detected from both 2007 and 2010 outbreaks. Real-time PCR is used as a supplementary diagnostic tool and RT-PCR and sequencing is conducted for genotyping.
The measles outbreak in 2007 involved 180 confirmed cases out of 451 suspected cases. About 45% of cases were infected by a nosocomial transmission and involved 81 cases from six hospitals, while 88% were under 2 years and unvaccinated. This outbreak taught the lesson that nosocomial transmission can play an important role in countries that already eliminated or are close to eliminating measles and timely vaccination is required to prevent such outbreaks. High-quality, laboratory-based surveillance with molecular detection would be critical. Intercountry collaboration would be critical to distinguish imported from endemic cases with or without strong epidemiological evidence in the country.

An outbreak in middle school in 2010 confirmed 78 cases by measles IgM or RT-PCR. Most of these cases did not have typical clinical signs of measles and paediatricians could not diagnose initial diagnosis of measles. Measles IgG testing was performed for paired sera from 14 cases with positive RT-PCR and negative measles IgM results. Increased IgG titers were detected from all 14 cases. Measles IgG avidity testing would be useful to interpret these cases.

In 2011, 16 clustering cases of measles were reported in Changwon city, Gyeongnam, where many foreign workers live. D9 strains were detected from all genotyped cases. This was the first detection of D9 strains in the Republic of Korea since genotyping was conducted in the country. He proposed to allocate clades or subgenotypes within the same genotype.

Molecular surveillance in US CDC

Dr Paul Rota from US CDC presented the experience of the Pan American Health Organization (PAHO) with molecular epidemiology of measles viruses and utility of molecular analysis for documentation of the verification of measles elimination. Molecular epidemiologic data are used as for the verification of measles elimination. A criterion for verification of elimination is absence of an endemic genotype for one year. Genotyping and sequencing can also be used to establish a genetic baseline for regional and global molecular epidemiology and elimination activities, to monitor virus transmission during and after outbreaks and to differentiate between ongoing transmission of endemic virus from new imported virus and import-related cases.

Genetic data, together with epidemiological information, can be used to track transmission patterns and identify the sources of infection. When endemic transmission of measles is occurring in a country, variations in nucleotide sequences with non-identical, distinct lineages are observed within the endemic genotypes. When a country is close to the elimination stage, no geographic or temporal pattern is presented for sporadic cases and multiple imported genotypes are detected with no endemic genotypes. However, reintroduction of identical or nearly identical measles viruses (with ~one nucleotide difference) following interruption of transmission can also happen and it would be not possible to differentiate with endemic transmission without epidemiological data. In 2010, PAHO countries experienced measles outbreaks after the Region of the Americas eliminated measles in 2000. The United States experienced four outbreaks related to three genotypes (59 cases). Canada and Brazil experienced five outbreaks (96 cases) and three outbreaks (66 cases) related to two and three genotypes, respectively. Argentina had an outbreak (17 cases) related to one genotype. In the United States, an outbreak is defined as more than three cases that are linked in time or place. From 2002 to 2011, 561 cases related to 272 chains of transmission from 40 outbreaks occurred in the United States. Thirty-one outbreaks (77%) and 102 chains of transmission (38%) had genotype information.

The last endemic measles genotype in the Americas was D9 in Venezuela in 2001. Molecular data supported evidence that the source was from outside of the Americas and was possibly imported from South-East Asia. Molecular epidemiological data collected between 2001 and 2011 show the absence of endemic genotype in Americas. In 2010-2011, D4, H1, D8, D9
and B3 genotype strains were detected in the Americas. D4 strains imported from France, Italy, Poland, Romania and United Kingdom were detected in the United States in 2011. In 2011, 198 cases from 15 outbreaks were detected in week 33. While the genetic analysis can confirm independent sources of infection if different genotypes or clearly distinct lineages are detected, molecular studies alone cannot differentiate between continuous circulation of virus and repeated importations from the same source.

2.3.5 Country and area reports

(1) Cambodia

The Immunology Unit at the National Institute of Public Health (NIPH) was designated as a NML and receives samples from all provincial referral hospitals and health centre sites. This laboratory conducts serological test using Siemens measles and rubella IgM ELISA kits. DBS are collected from children younger than 1 year of age and serum samples from children older than 1 year of age. From 2009 to July 2011, 9715 serum and DBS samples were tested. Among those samples, 809 (8.3%) were positive for measles and 1585 (16.3%) were positive for rubella. Increased rubella activity was noted in 2009 (13%) and 2011 (33%). An increase in measles cases was also detected in 2010 (16.4%). The laboratory has implemented the use of in-house control and confirmatory testing by Hong Kong (China) RRL as quality assurance measures as recommended by WHO. The proficiency testing score was 100% for measles and 95% for rubella in 2009-2010. Confirmatory testing scores were 97.1% in 2009, 95.7% in 2010 and 86.7% in 2011. Improvement of the collection and transport of samples for measles/rubella diagnosis, development of improved standardized measles/rubella database to meet laboratory needs, and refresher training of staff on testing, biosafety and biosecurity were presented as requests to WHO or areas for improvement.

(2) Fiji

The laboratory in the Mataika House was designated as the NML in Fiji. This laboratory receives samples from three sub-divisional hospitals/health centres and pathology laboratories in the Colonial War Memorial Hospital, Lautoka Hospital and Labasa Hospital. DBS and serum samples are tested in the laboratory. Siemens IgM ELISA kit is used for the serologic diagnosis of measles and rubella. From 2009 to July 2011, 238 serum samples were tested, with 6 (2.5%) testing positive for measles and 27 (11.3%) for rubella. The results of confirmatory testing (samples sent to VIDRL Australia) showed 85% concordance in 2010 and 91% concordance in 2011. The results of proficiency testing were 100% for both measles and rubella in 2010. From rubella outbreaks in 2011 in Fiji, 196 samples (149 males and 47 females) were tested and 57 (49 males and 8 females) were rubella IgM positive. Kit positive and negative control samples were monitored as recommended, but due to the lack of positive cases, in-house control samples could not be prepared and used.

(3) The Lao People’s Democratic Republic

The Lao People’s Democratic Republic has single-dose measles vaccination at 9–11 months and additional SIAs are regularly conducted to provide additional doses of measles vaccine. Routine vaccine coverage rate was 64% in 2010, and 81% of districts have coverage <80%. Acute fever and rash is one of 17 notifiable diseases in the Lao People’s Democratic Republic, and any case of acute fever and rash should be reported by provincial and district surveillance. The Virology section at the National Centre for Laboratory and Epidemiology (NCLE) serves as NML in the Lao People's Democratic Republic. NCLE has 10 laboratory technicians, three laboratory heads and two doctors. Once specimens are received in NCLE, it provides feedback within 48 hours. The ELISA method is used for detecting measles and rubella IgM antibodies with Siemens kits.
Although NCLE does not have capacity of molecular testing/genotyping and virus isolation for measles and rubella, equipment for molecular work such as conventional PCR, real-time PCR and luminex PCR are available. Starting from 2007, measles IgM negative samples have been also tested for rubella IgM. In 2009, 223 cases were tested for measles and rubella IgM and 27 and 42 were positive for measles and rubella IgM, respectively. In 2010, samples from 85 cases, which accounted for only 26% of suspected cases, were tested in NCLE. Between 2009 and July 2011, 383 cases were tested and 43 (11.2%) and 82 (21.4%) were positive for measles and rubella, respectively. The laboratory obtained WHO proficiency testing scores of 100% for measles testing and 95% for rubella testing in 2010. A proportion of samples were sent to Hong Kong (China) RRL for confirmatory testing in 2010-2011. The concordance rates were 100% for measles and 90% for rubella. However, the use of in-house control samples for ELISA has not been implemented yet. Genotype H1 measles strains were endemic in the Lao People’s Democratic, but recently D9 strains have also been circulating in the country. The timeliness and completeness of surveillance and laboratory reporting have been improved to 100% in 2011. The laboratory is implementing recommendations from the last on-site review to be fully accredited by WHO.

(4) Macao (China)

The routine immunization programme provides two doses of measles–mumps–rubella (MMR) vaccine at 12 and 18 months. The coverage rates were 90.8% for MMR1 and 88.1% for MMR2 in 2009. The Public Health Laboratory was designated as the NML in Macao (China) and the laboratory has the capacity to perform serology, viral culture and molecular techniques. From 2009 to 2010, a total of 1161 serum samples were tested for measles IgM from clinical and survey cases, and the positivity rate was less than 1%. During this period, rubella IgM and IgG tests were also performed on 8276 cases and 8352 cases, respectively. The rubella IgM positivity rate was less than 1%, and rubella IgG positivity was high at 92%. A measles survey programme in Macao (China) was initiated in 2002. About 500 samples were randomly selected from routine diagnostic specimens of 0 to >40 years old and tested for both measles IgM and IgG. Based on the results of the measles survey, the average measles IgG positive rate from 2002 to 2011 was 79%, ranging from 71% to 90%, which was lower than the expected value. The measles IgG positive rate in 2011 was 81%. For the quality assurance of testing, internal quality control samples have been used with Levey Jennings Quality control chart and interpretation, and the laboratory participated in various external quality assessment programmes. The results of WHO measles/rubella proficiency testing were 100%. Confirmatory testing samples were sent to Hong Kong (China) RRL and concordance of results was 100%. Additional genotyping using serum samples was conducted in Hong Kong (China) from 2009 to 2010, and H1 and D9 strains were detected. It was suggested that the recent measles D8 strain detected might have been imported from mainland China.

(5) Malaysia

Since 2004, two doses of MMR vaccine are given to children at 12 months and 7 years in Peninsular Malaysia and Sarawak. In Sabah, the first dose of single measles vaccine is given at 6 months followed by two doses of MMR vaccines given at 12 months and 7 years. Reports of coverage with the first dose of measles-containing vaccine (MCV1) from 2008 to 2010 show that the number of the districts with >95% coverage dropped from 67.6% in 2008 to 49.6% in 2010, and that 16.3% of districts showed 50%–80% coverage in 2010. The National Public Health Laboratory (NPHL) was designated as the NML for Malaysia, and the serology laboratory, cell culture laboratory and molecular laboratory in the Virology Division of NPHL are involved in WHO NML activities. Siemens Enzygnost IgM and IgG ELISA kits are used. Samples are tested for measles IgM first and then negative samples are tested for rubella IgM.
Table 2. Summary of laboratory testing for measles and rubella, 2009–2011, Malaysia

<table>
<thead>
<tr>
<th>Type of test</th>
<th>2009 No. Samples tested</th>
<th>2010 No. Samples tested</th>
<th>2011 (until July) No. Samples tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles IgM</td>
<td>2269</td>
<td>953</td>
<td>2276</td>
</tr>
<tr>
<td>No. Positive (%)</td>
<td>56 (2.5)</td>
<td>66 (6.9)</td>
<td>532 (23.4)</td>
</tr>
<tr>
<td>Rubella IgM</td>
<td>3184</td>
<td>935</td>
<td>1732</td>
</tr>
<tr>
<td>No. Positive (%)</td>
<td>1343 (42.2)</td>
<td>121 (12.9)</td>
<td>228 (13.2)</td>
</tr>
<tr>
<td>Measles Virus Isolation</td>
<td>124</td>
<td>98</td>
<td>1020</td>
</tr>
<tr>
<td>No. Positive (%)</td>
<td>1 (0.8)</td>
<td>2 (2.1)</td>
<td>37 (3.6)</td>
</tr>
<tr>
<td>Measles PCR</td>
<td>0</td>
<td>15</td>
<td>640</td>
</tr>
<tr>
<td>No. Positive (%)</td>
<td>0</td>
<td>1 (3.8)</td>
<td>124 (19.4)</td>
</tr>
</tbody>
</table>

Between 2010 and July 2011, 3229 samples for measles and 2667 samples for rubella IgM were tested, and positive ratios were 18.5% and 13.1%, respectively (Table 2). During this period, 1242 samples were processed for virus isolation and measles virus was isolated from 39 samples (3.2%); 655 samples were tested for PCR and measles virus was detected from 125 samples (19.1%). Samples for confirmatory testing were sent to PHLC Hong Kong (China) and concordance rates were 100% for both measles and rubella in 2010. In-house controls have been well implemented and proficiency tests results of 100% were obtained. Additional genotyping of samples from this laboratory was conducted by Hong Kong (China) RRL, and measles genotypes G3, B3, D9 and A (vaccine-related) and rubella genotype 2B strains were detected in 2010-2011. The quality of testing done by the public health laboratory in Sabah, which has been involved in measles testing, needs to be monitored.

(6) Mongolia

Measles single-dose vaccination was introduced in 1973, and MCV2 was introduced in 1986 in Mongolia. In 1994, subnational immunization days for measles vaccination were conducted for children aged 3–7 years in selected areas. In 1996 and 2000, National Immunization Days were conducted for children aged from 9 months to 8 years and from 9 months to 10 years, respectively, and measles vaccine coverage rates reached 97%. The last outbreak of measles occurred in Mongolia during 2001–2002. Rubella vaccine was introduced in 2001 in Ulaanbaatar city for children aged 3–17 years old and women aged 18–34 years, and vaccination rates reached 96.6% and 86.7%, respectively. In 2007, National Immunization Days for measles vaccination was conducted for children aged 10 months to 10 years. MMR vaccination started in 2009. Coverage in 2010 was 97% for MMR1 and 95% for MMR 2. The National Centre for Communicable Diseases serves as NML in Mongolia. The laboratory has the capacity to perform serology, virus culture using Vero/SLAM cells and PCR methods. For serology, Siemens IgM and IgG ELISA and Indirect Immunofluorescence Assay (IFA) are used. From 2010 to August 2011, 256 serum samples were tested; all samples were negative for measles and 21 samples (8.2%) were positive for rubella. The last measles case was detected in 2009. The laboratory conducted mumps diagnosis from 2009 to 2011, and the mumps IgM positive rate was very high at 64%. Parvovirus B19 diagnosis was also introduced in 2011. Two samples were tested and were IgM positive. To obtain baseline measles genotype information in Mongolia, measles IgM positive serum samples were sent to Hong Kong (China) RRL for genotyping. Genotype D6 strains were detected from 2006 samples, and H1 strains were detected from 2008 and 2009 samples. From rubella IgM positive serum samples, 1E genotype strains
were detected. In 2011, mumps outbreaks occurred in Mongolia, and samples from the outbreaks were sent to Hong Kong (China) RRL for genotyping. Mumps F genotype strains were detected by Hong Kong (China) RRL. Mumps H3 genotype strains were also detected from another outbreak in 2011. Confirmatory testing samples from 2000 to 2011 were sent to Hong Kong (China) RRL. Concordance rates of >90% were observed in 2010. In 2011, 100% concordance rates were observed for both measles and rubella. To send out confirmatory samples, the laboratory needs to obtain permits from the Ministry of Health and State Professional Inspection Agency.

(7) New Zealand

The Canterbury Health Laboratory serves as NML for New Zealand. The laboratory conducts IgM and IgG ELISA detection, cell culture and molecular diagnosis of measles and rubella viruses. Parvovirus B19 detection is included in the testing strategy as differential diagnosis of measles IgM positive samples from referring laboratory. This laboratory receives serum, throat or nasopharyngeal swab and urine samples for ELISA, virus culture and PCR. For measles IgM ELISA, Siemens kit is used, but for rubella IgM test, Biomerieux MiniVidas kit is used. For measles and rubella IgG tests, Biomerieux MiniVidas and Euroimmune kit are used, respectively. In 2009, there were three measles outbreaks affecting mainly Southland, Canterbury and Auckland, accounting for a total of 205 cases. The majority of the cases were part of a large outbreak in Canterbury, which started while pandemic influenza A(H1N1) was still ongoing. In March 2010, a large measles outbreak occurred in Northland in a low-immunization area. The index case of this outbreak was a person infected in India. A total of 31 cases were identified during this outbreak, and cases spread to other areas of New Zealand. In 2011, three importations caused by D9 and D4 strains occurred from January to August. D9 importations into Auckland, Wellington and Christchurch were followed by D9 importation to Hawkes Bay and D4 importation to the Auckland area. Age analysis of measles cases in 2011 showed the peak in children aged 5–9 years followed by 10–14, 1–4 and 15–19 years. Among 188 cases in 2011, 153 cases were not vaccinated, 17 had only one dose and 63% were European ethnicity. A small rubella outbreak also occurred in 2011 in Auckland among non-immunized people. Two adults had recently travelled to Fiji where a rubella outbreak was ongoing. This laboratory participated in various external quality assurance programmes and implemented in-house controls for serological testing.

(8) Papua New Guinea

The Central Public Health Laboratory at Port Moresby was designated as NML for Papua New Guinea. IgM ELISA testing is performed with Siemens kits supplied by WHO. Measles and rubella testing is performed in parallel. This laboratory participated in a WHO proficiency testing and confirmatory testing programme for quality assurance and was recently accredited by WHO. Fifty-six samples including WHO proficiency panel samples were tested in 2010, and a total of 44 samples were tested from January to July 2011. All samples were negative for measles in 2010 and 2011, and 28 samples (38.6%) were positive for rubella in 2011. In 2009, the results of proficiency testing by VIDRL were 90% for measles and 100% for rubella. In 2010, the laboratory scored 100% for measles PT, 95% for rubella PT and 100% for confirmatory testing in VIDRL.

(9) Philippines

The Virology Department, Research Institute for Tropical Medicine (RITM) was designated as NML in the Philippines. This laboratory is capable of performing serology and cell culture. Hong Kong (China) RRL conducts the molecular detection for measles and rubella genotyping for the Philippines. From 2009 to July 2011, a total of 15 861 samples were tested for
measles serology. Of these, 7013 (44%) were positive for measles IgM. During this period, rubella serology was also performed on 9348 samples, which were negative or equivocal for measles. Thirty-two per cent of samples were positive for rubella IgM. Big outbreaks of measles occurred in 2010 and 2011. The majority of measles cases in 2010-2011 were from the National Capital Region (NCR) among children under 2 years old. In 2011, measles outbreaks also occurred in other regions, including regions 1, 3, 4A, 5 and 11. A measles/rubella SIA was conducted from 4 April to 31 May 2011. This door-to-door campaign covered children aged 9–95 months.

It was noted that 38% and 45% of samples received in 2010 and 2011 were reported within 7 days, respectively. For the quality assurance, confirmatory testing samples were sent to VIDRL Australia in 2009 and to Hong Kong (China) RRL in 2010. Concordance rates of 2009 and 2010 samples were 96.7% and 98.7% for measles and 100% and 97.7% for rubella, respectively. This laboratory obtained 100% for WHO measles and rubella PT in 2009 and 2010. The use of in-house control samples was well implemented for measles and rubella IgM assays, and the long-term trend of OD values of in-house control samples was monitored. Additional genotyping results were obtained from Hong Kong (China) RRL using IgM positive or equivocal serum samples. Measles D9 strains from regions 7, 12 and NCR and G3 genotype strains from region 6 were detected from 2009 samples. This laboratory received virus isolation samples from 2010. In 2010, 35 measles and 3 rubella isolates were obtained out of 127 virus isolation samples received. In 2011, 30 measles and 1 rubella isolates were obtained out of 166 virus isolation samples received. Viral isolates were sent to Hong Kong (China) for genotyping, and D9 measles and 1j and 2B rubella strains were identified (Figure 1)

Figure 1. Regional distribution of measles and rubella genotypes, 2009–2011, Philippines

(10) Republic of Korea

The NML in Korea CDC performs serology, virus isolation and molecular detection using real-time PCR and sequencing for confirming measles and rubella infection.
diagnosis for parvovirus B19 and Human Herpes Virus 6 (HHV 6) are also performed using Biotrin IgM EIA (Parvovirus B19) and Panbio IgM EIA (HHV 6). In 2009, two imported measles cases were identified and genotypes B3 and H1 strains were identified. An outbreak of measles occurred in Incheon in 2010, with 78 samples (46.4%) positive either by IgM ELISA or PCR. From this outbreak, 43 RT-PCR-measles-positive samples were confirmed as H1 strains. From outbreak cases in 2011 in Gyungnam province, where many foreign workers live, D9 strains were detected from all RT-PCR-measles-positive cases 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 the 2010 outbreak, 14 measles cases were IgM negative and were confirmed by RT-PCR. The results of the differential diagnosis showed that true measles IgM positive cases were at most 42% of suspected measles cases and more than 20% were HHV-6 or Parvo-B19 IgM positive cases. It was emphasized that discrimination of HHV-6 and Parvo-B19 infection should be investigated to confirm measles from suspected cases, especially among children under 5 years old.

Table 3. Summary of laboratory testing for measles, Korea CDC, 2008–2011

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Virus isolation</th>
<th>IgM positive</th>
<th>IgG increase</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2009</td>
<td>32</td>
<td>0</td>
<td>31</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2010</td>
<td>91</td>
<td>20</td>
<td>44</td>
<td>4</td>
<td>43</td>
</tr>
<tr>
<td>2011.8</td>
<td>34</td>
<td>1</td>
<td>31</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>21</td>
<td>128</td>
<td>0</td>
<td>57</td>
</tr>
</tbody>
</table>

This laboratory is also responsible for external quality assurance of provincial health and environmental research institutes (PHERIs). It sends quality assurance panel samples for serology and RT-PCR to PHERIs and five major private diagnostic centres. The panel samples, composed of two measles positive, four rubella positive and four negative serum samples, were validated and reconfirmed in VIDRL before the distribution. In 2011, panel samples were tested in 11 PHERIs by Siemens ELISA IgM kits and in 5 private diagnostic centres by Radim (2 centres), Euroimmunne (2 centres) and Biorad (1 centre). All participating laboratories reported good results. In 2011, RT-PCR panel samples, which were prepared from measles, rubella and mumps isolates in the Republic of Korea (2 measles, 1 rubella and 1 mumps) were also distributed to PHERIs. All participating laboratories reported the final results within 35 days after the receipt and received good scores.

(11) Singapore

A two-dose MMR vaccination programme was implemented in 1998. A national serosurvey in 2005 showed an overall seropositive rate of 96.7% for measles and 87.4% for rubella among adults aged 18–74 years old. It was noted that 15.8% of 18–44 year old females remain susceptible to rubella infection. An increased incidence of rubella was noted in 2009 and 2010, while an increase in measles cases occurred in the first quarter of 2011. The Virology
Laboratory in the Pathology Department of Singapore General Hospital serves as the NML and performs serology as well as virus isolation, direct antigen detection and virus genotyping. Siemens Enzygnost kits are used for measles and rubella IgM detection. Testing algorithm is decided based on clinician's request (Figure 2a).

Figure 2a. Laboratory testing algorithm for measles and rubella in Singapore

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A new active surveillance initiative has been set up to improve the measles surveillance system in Singapore. With the new initiative, adult cases will be tested by IgM ELISA in NML and PCR testing of all suspect cases (adult and children) will be conducted by the National Public Health Laboratory (NPHL). Subsequently, NPHL would refer a proportion of PCR positive samples to NML for measles genotyping (Figure 2b).

Figure 2b. Laboratory testing algorithm for active measles surveillance (new initiative) in Singapore
This laboratory participates in various external quality assurance programmes including WHO accreditation. All technical staff are trained and competency is assessed before performing laboratory tests. Quality control data are assessed and monitored for each run, and results are assessed and validated before reporting. Monitoring and maintenance of equipment are regularly performed. WHO PT results were 100% in 2009-2010, and confirmatory testing by Hong Kong (China) RRL showed good concordance between two laboratories from 2009 to 2011. From 2009 to 2011, 526 samples were tested for measles IgM and 1399 samples were tested for rubella IgM. Genotyping was performed on 52 samples. The results showed H1, D8 and D9 strains in 2009; H1, G3 and D9 strains in 2010; and G3, D4, D8 and D9 strains in 2011. The common circulating measles strains belong to the genogroup D.

(12) Viet Nam

NIHE in Hanoi and Pasteur Institute in Ho Chi Minh City serve as the National Measles and Rubella Laboratory for northern and southern Viet Nam, respectively.

NIHE: A huge measles outbreak occurred in 2009. Of the 3890 samples tested, 56% were positive for measles IgM and 25% were positive for rubella IgM. In 2010 and 2011, 978 and 1364 samples were tested for measles IgM, and 28% and 1% were positive for measles IgM, respectively. The laboratory used Siemens and Biorad kits in 2009 and 2010 and Siemens and IBL kits in 2011. In 2010 and 2011, increased rubella cases were detected. Among 1130 and 2353 cases tested for rubella IgM, 44% and 58% were positive for rubella IgM, respectively. Timeliness of testing was affected in 2011. Among 2353 samples tested in 2011, 39% were tested and reported after 7 days. Quality assurance measures including the use of in-house control samples and confirmatory testing in Hong Kong (China) RRL were well implemented. The laboratory has obtained a 100% PT score since 2006, and confirmatory testing results also showed good concordance from 2007 to 2010. Virus isolation and molecular detection of measles and rubella have also been performed since 2006. From 2006 to 2011, 48 samples (19.4%) out of 248 throat swab samples showed cytopathic effects. From 2006 to 2009, H1 measles genotype strains were detected and rubella 2B strains were detected in 2009 and 2011 in northern Viet Nam.

Pasteur Institute, Ho Chi Minh City: The MCV2 vaccination at 6 years old was introduced in 2006, but in 2010-2011, the age of MCV2 vaccination was changed to 18 months after a huge measles outbreak. From September to November 2010, a national vaccination campaign for children under 6 years old was conducted. The laboratory confirmed a huge number of measles cases (n=2717) in 2009. In 2010 and 2011, decreased numbers of measles cases were detected: 885 cases in 2010 and 5 cases in 2011. However, increased rubella activity continued in 2010 (n=1833) and 2011 (n=1059) after 1028 cases were detected in 2009. This laboratory has obtained a 100% score for PT since 2007, and confirmatory testing results also showed good concordance rates of 100% for measles and rubella. CRS surveillance in southern Viet Nam will be also implemented.

(13) Challenges of measles and rubella laboratories

<table>
<thead>
<tr>
<th>Country</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambodia</td>
<td>• Quality of some samples is poor (e.g. haemolysis and DBS)</td>
</tr>
<tr>
<td></td>
<td>• Inadequate volume of sample</td>
</tr>
<tr>
<td></td>
<td>• Timing of sample collection and sending the samples to NML</td>
</tr>
<tr>
<td></td>
<td>• No standardized database system</td>
</tr>
<tr>
<td>Country</td>
<td>Issues / Actions</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Fiji                         | • Lack of human resources  
• Local Networking – information sharing  
• Laboratory reports not reaching clinicians  
• Insufficient volume of some samples  
• Equipment maintenance for ELISA washer, biosafety cabinets  
• Test kits in case of outbreak  
• Achieving requirements for accreditation |
| Lao People’s Democratic Republic | • Relocation to new premises due to refurbishment of NCLE building  
• Improvement of data management and coordination with other units  
• Lack of training of provincial and district staff on field investigation and collection of blood specimens/swabs  
• Laboratory biosafety issues  
• Procurement of kits (should procure 3 months in advance and keep stocks enough for 6 months period at all times) |
| Macao (China)                | • Imported measles cases due to the tourism  
• The positive rate of measles IgG in survey samples is lower than expected value  
• False positive cases in rubella IgM test  
• Change of laboratory testing system: Rubella is tested together with TORCH items)  
• ISO 15189 accreditation  
• Laboratory automation |
| Malaysia                     | • Incomplete clinical data  
• Quality monitoring on subnational laboratory  
• Improvement in lab data (genotyping) for timely submission to WHO (via MeaNS)  
• Deposition of rubella genotyping (currently via genebank) |
| Mongolia                     | • Improvement of infrastructure of National Measles Laboratory.  
• Improvement of laboratory testing quality  
• Organizing on-the-job training of laboratory technicians with the assistance of WHO laboratory consultant |
| New Zealand                  | • Comprehensive and complete data set collection because many other laboratories are capable of diagnostic testing  
• Issue with collection of data and samples for confirmatory testing  
• Centralizing the national data set |
| Papua New Guinea             | • Minimal request of testing for measles/rubella and AFP  
• Biosafety training for CPHL staff, provincial laboratory managers and disease control officers  
• Training on shipment (packaging and transport) of infectious substance for provincial laboratory managers and disease control officers  
• Availability of skim milk for processing of DBS |
| Philippines                  | • Drafting of Administrative Order (AO) for enhanced surveillance after the 2011 measles/rubella SIA; use of DBS for selected areas; guidelines for nasopharyngeal swab (NPS) collection for clustering of |
cases
- Measles-rubella case investigation form (CIF) currently in use; need to prepare CIF for rubella-CRS surveillance
- Incompletely filled measles case investigation forms and incomplete data
- Specimen shipment in reverse cold chain practices
- Enhancement of laboratory capacity (additional manpower) to perform additional diagnostic tests like Immuno Colorimetric Assay and PCR
- Mycoplasma contamination of VeroSLAM cell lines.
  Data management issues: unable to utilize WHO MS Access database pending customization to address local needs

| Republic of Korea | Training of staff in PHERIs for laboratory diagnosis of measles and rubella
| Singapore        | WHO algorithm for measles and rubella testing
|                  | Non-NML laboratory-confirmed cases
|                  | Little clinical and epidemiological information is available, and it is difficult to provide this information to WHO.
|                  | Monthly data report: integration of Excel format into Access format

### 2.3.6 Use of alternative sampling

1. Use of dried blood spot (DBS) and oral fluid (OF) samples

   Mr David Featherstone presented the usefulness and stability of DBS and OF samples, which were fully evaluated as alternative samples to serum. For DBS samples, it is recommended that four full circles are fully filled and dried at least for one hour before shipping. For each test, three discs from each circle are needed. For OF collection, the ORACAL saliva collection device produced by Malvern Medical Developments, Ltd has been widely used among network laboratories. Extraction methods from OF samples for serology and RT-PCR have been presented. Plasma-derived IgM in OF samples was evaluated by the Health Protection Agency, and other network laboratories participated in a field assessment of the OF collection method for measles and rubella IgM ELISA assay. Measles virus genome can be detected from OF samples for up to 28 days post onset. Based on comprehensive validation, OF samples provide a unique opportunity to enhance molecular surveillance as well as provide case confirmation for suspected measles cases. For ELISA assay of OF samples, Microimmune IgM ELISA kit should be used. Quality assurance programmes for DBS and OF need to be established.

### 2.3.7 Strengthening rubella virus surveillance and the role of the laboratory

1. Improving rubella virus surveillance

   Dr Joseph Icenogle from US CDC presented molecular techniques for rubella virus detection and some practical considerations to improve rubella virus surveillance. Since time course for the presence of the virus and time course for the presence of immunoglobulin to rubella virus are different, serological and molecular detection of diseases have different sensitivities of detection after the disease onset. Molecular methods can be used as an additional
confirmatory method during the approach to elimination by providing tracking of strains and aiding in the classification of usual or severe cases or confirming suspected vaccine reactions. One of the challenges for rubella surveillance is that many postnatal rubella cases are IgM negative in the first few days after rash onset. In some countries, the majority of the specimens are taken within the first week of rash onset; with specimens collected on the first three days of rash onset, more will be RT-PCR positive than IgM positive. With specimens collected on the fourth day of rash onset and later, IgM will confirm the majority of cases. More than 80% of suspected rubella cases were confirmed with combined results of molecular and serologic testing in the first four days after rash onset. When the incidence of measles, rubella and CRS infections are low in an elimination setting, the positive predictive value of specific IgM testing for confirming disease is also low. Rubella virus isolation or detection of viral genomes by RT-PCR can also be used to classify suspected CRS cases. RT-PCR and real-time PCR can be the methods of choice for rubella detection due to various reasons: (1) rubella virus does not produce the cytopathic effect; (2) RT-PCR is easier to perform than virus isolation for rubella; (3) almost all CRS patients are positive by RT-PCR at birth; and (4) rubella virus RNA can be detected directly from clinical samples without amplification in cell culture due to high virus titer. Urine, throat swab and nasopharyngeal secretions are the most reliable specimens for rubella RT-PCR and real-time PCR for CRS surveillance. US CDC established rubella diagnostic PCR with three primers, and real-time PCR for rubella has also adopted a three-primer RT-PCR protocol to detect 10 different genotypes. RT-PCR reactions with three primers can amplify one single amplicon and two amplicons of fragment 1 and fragment 2. Genetic characterization has identified 2 clades that differ by 8%–10% at the nucleotide level. Clade 1 is divided into 10 genotypes, of which six are recognized (1B, 1C, 1D, 1E, 1F and 1G) and four are provisional (1a, 1h, 1i and 1j). Clade 2 contains three genotypes of 2A, 2B and 2C. Genotypes 1E, 1G and 2B are widely distributed and frequently detected, and 1E, 1j and 2B are circulating in the Western Pacific Region. During the training workshop in Hong Kong (China) RRL in 2010, nine laboratories received FTA practice panel samples, and most laboratories successfully completed real-time PCR and genotyping.

(2) Rubella and congenital rubella syndrome surveillance in China

Dr Zhen Zhu presented rubella epidemiology, rubella virus and CRS surveillance in China. A rubella outbreak occurred in 2008 with an incidence of 9.1 per 100 000 population (120 354 cases). The incidence of rubella has been higher than measles since 2009. During 2004–2011, rubella cases were reported throughout the years but peaked between March to June and in December. The proportion of cases between 15 and 39 years of age has increased since 2004, which could be a concern for rubella cases of women of child-bearing age leading to CRS. A flowchart that shows how rubella virus is identified from clinical samples at the provincial laboratories and China CDC was presented. At the provincial laboratories, virus isolation and real-time PCR is conducted in parallel. If real-time PCR is positive, then RT-PCR and virus isolation are conducted and sequencing is performed for RT-PCR positive samples. Rubella isolates are transferred to China CDC. If provincial laboratories determine rubella virus sequences, sequence information will be sent to China CDC. China CDC also receives virus isolates from provinces and conducts real-time PCR, RT-PCR and sequencing. Sequencing results are sent to the WHO Regional Office on a monthly basis. During 1999–2011, 1057 rubella virus isolates were detected from 27 provinces, and during 2010–2011, 348 isolates were detected from 19 provinces. Out of 1057 rubella isolates from 1999 to 2011, 471 from 24 provinces were genotyped. From 2010 to July 2011, 208 isolates from 16 provinces were genotyped. During 1999–2011, four rubella virus genotypes were detected: 1E (n=419), 1F (n=15), 2A (n=3) and 2B (n=34) The rubella virus detected during this period was predominately 1E strains. While 1F and 2A strains have not been detected since 2002 and 2001, respectively, 2B strains were newly detected in 2006. Rubella virus strains have not been detected from seven provinces: Xinjiang, Tibet, Yunnan, Guizhou, Hubei, Shaanxi and Beijing. While 1E strains are
predominantly circulating throughout China, 2B strains have been detected in a few provinces including Shandong and Hainan. She also presented the phylogenetic analysis of 1E strains detected in China showing three different groups. Lineage 1 of 1E strains was the predominant lineage in China and has not been found outside of China. She proposed the theory that this may due to mutations conferring a selective advantage of this lineage following its introduction in 2001 or multiple importations from unknown location. On the other hand, the 2B virus found in 2000 continuously circulated throughout 2008, but some surveillance gaps may exist in China.

In 2009, a three-year Ministry of Health-WHO project to establish prospective rubella and CRS surveillance has been initiated in two cities in Shandong (Jinan and Yantai) and Heilongjiang (Qiqihaer and Haerbin) provinces. This project involved investigation and follow-up of pregnant women and suspected CRS cases in close collaboration with the birth defect surveillance system. This project also involved developing the full investigation of rubella outbreaks, the study of rubella vaccine efficacy as well as surveillance of rubella vaccine immunization coverage.

(3) Rubella and CRS surveillance in Japan

Dr Katsuhiro Komase from NIID presented rubella and CRS surveillance in Japan. Japan introduced routine vaccination of rubella vaccine targeting junior high school girls aged 12–15 years old. MMR vaccination was introduced to children aged 12–90 months between 1989 and 1993. In 1995, routine rubella vaccination was introduced to children 12–90 months old and a two-dose schedule of MR vaccination was introduced in 2006. During 2008–2012, in addition to routine MR vaccination for 1-year-old children and children who are 6–7-years old (one year before primary school entry), supplementary MR vaccination targeting children 12–13 years and 17–18 years old was also conducted. In 2010, the coverage of MR vaccination was 95.6% for the first dose, 92.2% for the second dose, 87.2% for the third dose and 78.8% for the fourth dose. Case-based reporting for rubella cases together with measles cases started in 2008. In 2004, 4339 cases of rubella cases and 10 CRS cases were reported based on sentinel surveillance. Since the introduction of new supplementary MR vaccination, rubella cases decreased to 90 in 2010. However, in 2011, 262 rubella cases were reported as of week 29. Outbreaks in 2011 mainly occurred among males 20–50 years old with low rubella antibody positive rate. The ratio of male to female cases was 3:1. Ten domestically infected CRS cases were reported in 2004, and three imported CRS cases were reported in 2005 (from India), 2009 (from Philippines) and 2011 (from Vietnam). Laboratory confirmation was mainly done by the IgM detection at the commercial laboratories. Proportion of laboratory-confirmed cases among reported rubella cases increased from less than 50% in 2008 to 75% in 2011). Among 30 rubella cases confirmed by PCR or virus isolation in 2011, seven 1E, one 1j and four 2B strains were detected. Some cases were considered as imported or import-related cases based on genome analysis and epidemiological link. Sequences were slightly different even in the same genotype, suggesting that viruses may have been brought from multiple sources and/or been circulating silently in Japan for a long time.

2.3.8 Reports from GSL and RRL

(1) Current status of measles in Japan

Dr Katsuhiro Komase presented the activities at NIID as WHO measles GSL and RRL. A collaborative study was conducted with NCLE and the WHO Country Office in the Lao People’s Democratic Republic entitled “Seroprevalence of anti-rubella among woman of child-bearing age”. The result of this study demonstrated that the seropositive rates for rubella IgG were 64.5% among 15–19 year olds, 80% among 20–24 year olds, 86% among 25–29 year olds and 92.3% among 30–35 year olds. From 31 January to 3 February 2011, NIID Japan conducted a hands-on training on Laboratory Diagnosis Techniques for the Control of Vaccine Preventable Diseases,
including poliomyelitis and measles, which was jointly hosted by JICA and NIID. This laboratory provided the measles and rubella IgM ELISA PT to 10 Measles/Rubella Reference Centers. It also provided Vero/SLAM cells and measles rubella reference RNA for PCR to the prefectural institutes of public health. Results of ELISA PT conducted in 10 Reference Centers showed that 9 out of 10 centres scored 100% and one centre scored 95%. In 2008, a special guidance for measles from the Ministry of Health, Labor and Welfare dated 28 December 2007 was issued to eliminate measles by 2012 and to maintain measles-free status onwards in Japan. With this initiative, SIAs targeting 12- and 17-year-old children for five years have been conducted from 2008. A case-based reporting system instead of a sentinel sites reporting system was also introduced, and measles cases have been confirmed at the early stage of disease to prevent secondary infection. In 2010, MR vaccine coverage for four doses including SIAs were 95.6% for the first dose, 92.2% for the second dose, 97.2% for the third dose and 87.2% for the fourth dose. After the introduction of measles SIA policy in 2008, measles incidence decreased in 2009 and 2010. The number of measles cases decreased by more than 95% from 2008 to 2010. The ratio of laboratory-confirmed cases to reported cases also increased in 2010 and 2011. Measles genotypes detected in Japan showed that no endemic strains were detected and mixed genotypes associated with importation were detected. Age distribution of measles cases in 2011 showed that only 38% of cases were under 10 years old and that 35% of cases were adults aged 20–39 years old. Efforts to establish an expert committee for measles diagnosis to lead accurate diagnosis towards the measles elimination goal in Japan remain to be a challenge. NIID developed an assay for the detection and quantification of the measles virus nucleoprotein (N) gene using rRT-PCR and this was compared to the US CDC protocol. The results of the comparison of the two assays showed that the US CDC method was more sensitive than the NIID method. Introduction of real-time PCR methods for accurate and rapid diagnosis is planned.

(2) Report from VIDRL Australia

Ms Jennie Leydon presented VIDRL’s activities as WHO measles/rubella RRL. In addition to preparing PT samples for global network laboratories, it conducted confirmatory testing for Fiji, Malaysia, New Zealand, Papua New Guinea, the Philippines and the Republic of Korea in 2009–2011. This laboratory also received samples from Fiji, New Zealand and Solomon Island for case investigations in 2009–2010. For measles and rubella IgM ELISA, For confirmatory tests, VIDRL used Bio-Rad Platelia (IgM capture) and Chemicon Light (IgM capture) diagnostics assays for measles and Beckman Access (IgM capture) and Diasorin assays for rubella in addition to Siemens assay. For measles IgM ELISA, the laboratory used an automated EIA processor, BEP 2000, which can perform tests for measles, mumps and varicella zoster simultaneously (Figure 3a). For rubella IgM testing, an automated chemiluminescent assay processor, Beckman Coulter, was used (Figure 3b). From 2009 to July 2011, 9141 tests for measles and 2357 tests for rubella were conducted at VIDRL. The rash illness-testing algorithm used in this laboratory involves measles IgG/IgM, rubella IgG/IgM and parvovirus B19 IgG/IgM testing. With this algorithm, cross-reactivity for IgM can be detected, and if the case is IgM positive for measles and rubella, IgM response could be due to recent MMR vaccination.
Figure 3a. Interpretation of results and testing algorithm of measles in VIDRL

Measles IgM/IgG performed on BEP 2000

- **IgM pos/IgG pos**: Recent infection
- **IgM pos/IgG neg**: Recent infection
- **IgM neg/IgG neg**: Request a follow-up sample (PCR if nose/throat swab not available)
- **IgM neg/IgG pos**: Past infection or immunization

**PCR**

This laboratory participates in ISO 15189 (NATA standards for accreditation) and Royal College of Pathologists of Australasia (RCPA) serology quality assurance programmes. Vaccine-preventable diseases module and antenatal serology module with two measles and two rubella samples are received two times or year times a year. Siemens kit is used in many laboratories in Australia for measles IgM testing, but other EIA kits such as Diesse, Vital and Euroimmune kits are also used. For rubella testing, Abbott CMIA, MEIA, Roche Diagnostics and Beckman Coulter

Figure 3b. Interpretation of results and testing algorithm of rubella in VIDRL

Rubella IgM/IgG performed on Beckman Coulter Access

- **IgM pos/IgG neg or IgM pos/IgG pos**: ? Recent infection
- **IgM neg/IgG neg**: Request a follow-up sample
- **IgM neg/IgG pos**: Past infection or immunization
- **IgM neg/IgG neg**: Request a follow-up sample
- **IgM pos**: Recent infection
- **IgM neg**: ? False positive
- **IgM neg**: Request a follow-up sample
kits are used. From January to July 2011, among 23 measles IgM positive cases, 21 (91%) were PCR positive. Genotypes identified were D8 (16 cases), D4 (5 cases), D9 (3 cases), H1 (1 case) and vaccine-related A (3 cases). Genotyping results were obtained using nose and throat swabs except for one case where serum was used. Rubella notification increased in 2010 and 2011 as compared to 2009. No case of congenital rubella was reported from 2009 to 2011. Confirmatory testing was performed for samples from Fiji, New Zealand, Papua New Guinea, the Philippines and the Republic of Korea. Results from 2009 to 2011 showed that most of the referring countries from the Western Pacific Region achieved >90% concordance results with VIDRL.

(3) Running status of measles laboratory network in China

Dr Yan Zhang presented on measles epidemiology during the period 2004–2011, measles SIA activities, results of measles serologic tests from outbreak and sporadic cases during 2010–2011 in China, virus surveillance and quality control of the measles laboratory network in China. In China, the migrant population is 0.26 billion, and 18.5% of the population is under 15 years old. From 2004 to 2009, province-specific measles SIAs were conducted in 27 out of 31 provinces (excluding Beijing, Shanghai, Henan and Heilongjiang) and high immunization coverage of average 97.3% was achieved. From 11 to 20 September 2010, a nationwide, synchronized SIA for measles targeted 105.1 million population and 97.5% were vaccinated. Reported measles cases dramatically declined to 73% in 2011 compared to 2010. From January to July 2011, an historically low total of 9258 measles cases were reported in China. During the period 2004–2011, the age distribution of majority measles cases shifted from 1–5 year old children to under 1-year-old infants. However, in March 2011, a measles outbreak occurred in Hotan prefecture of Xinjiang province. A total of 1423 cases were reported in Hotan with the incidence rate of 66.8 per million. Age distribution of cases showed that 43% were under 1 year old, 40% were 1–14 years old and 17% were >15 years old. Genotype confirmation was performed using 24 throat swab samples collected from four counties of Hotan prefecture. H1 strains with 100% homology were detected. In 2009, a single comprehensive measles surveillance information system was established combining national EPI and laboratory databases, and performance indicators have been progressively improved during the period of 2004–2011. In 2010 and 2011 (as of July), 41 123 and 23 628 samples were collected from sporadic cases out of 53 684 and 26 584 suspected cases showing 76.6% and 88.88% sample collection rates, respectively. The measles IgM positive rates of samples from sporadic cases in 2010 and 2011 were 60.4% and 30.7%, respectively. From outbreak cases, the serum sample collection rates were 94.9% in 2010 and 100% in 2011, and virus isolation sample collection rates were 40.2% in 2010 and 79.3% in 2011, respectively. It was noted that the collection of virus isolation samples has greatly increased in 2011. Timeliness of sample collection and transportation remains as one of the challenges in China.

(4) Report from Public Health Laboratory Center, Center for Health Protection (CHP) as WHO national measles laboratory and regional reference laboratory in Hong Kong (China)

Dr Janice Lo presented laboratory activities as NML and RRL in Hong Kong (China). For laboratory testing of measles, ELISA (Microimmune and Siemens kits), complement fixation, virus isolation using Vero/SLAM cells, IFA using mouse anti-measles nucleoprotein monoclonal antibody (Chemicon) and RT-PCR followed by sequencing targeting 545bp of N gene are performed. Measles surveillance data from 2006 to 2011 showed that the number of measles cases notified by CHP decreased from 106 cases in 2006 to 13 cases in 2010. Measles virus genotyping data showed that H1 strains, which were detected as endemic strains from 2006 to 2008, were detected less often in 2009 (5) and 2010 (1) and have not yet been detected in 2011 (0). In addition, the laboratory detected one B3 strain in 2010, one D4 strain in 2006, two D8 strains in 2010 (1) and 2011 (1), and two D9 strains in 2010 (1) and 2011 (1). Thirteen vaccine strains (A) were detected annually from 2006 to 2010. For rubella testing, ELISA (Siemens and
VIDAS kits), haemagglutination inhibition assay, virus isolation, IFA using mouse antirubella E1 envelope protein monoclonal antibody (Chemicon) as well as RT-PCR targeting 103bp of the E1 gene are performed. Rubella surveillance data from 2006 to 2011 showed that the number of rubella cases notified by CHP was lower than 50 per year (34–45). Rubella genotyping data showed that IE strains have been continuously detected from 2008 to 2011, while 1j and 2B strains were also detected during 2008–2011. A seroprevalence study was conducted using IgG ELISA and plaque reduction neutralization test (PRNT) for measles and single radial haemolysis and IgG ELISA for rubella. The results showed a high level of immunity against measles (>95% for all age groups except 94% for 1–4 years old). For rubella, >90% seropositive rates for all age groups except 35–39 year olds (82%) in 2009.

As a WHO measles RRL, this laboratory provided confirmatory testing and genotyping for many countries in the Region and hosted WHO hands-on training workshops. Confirmatory testing for measles and rubella were performed for nine network laboratories from the Region and results showed good concordance rates of >90% for most network laboratories. Virus isolation samples were referred from Cambodia, Lao People’s Democratic Republic, Macau (China) and the Philippines in 2008–2010. This laboratory also conducted genotyping using serum samples positive or equivocal for measles IgM, and the genotyping data obtained from serum samples provided valuable background genotype information on circulating measles virus strains in the Region. Rubella genotyping was also conducted for IgM positive or equivocal samples and 1E, 1j, 1h and 2B strains were detected. Insufficient volume of serum samples for confirmatory testing or genotyping, inadequate clinical/epidemiological information for test interpretation and further testing and insufficient epidemiological data for selection of rubella genotyping using serum samples were presented as challenges for the laboratory.

2.3.9 Measles serosurvey

(1) Standardization of measles serosurvey

Mr David Featherstone presented challenges in standardization of measles serosurveys. He discussed the functions of serosurveys such as finding out population immunity following a vaccination campaign, monitoring subgroups of a population and determining susceptibility profiles. He also discussed if a serosurvey would be the best method and if there are other ways to monitor population immunity. To monitor population immunity, immunization coverage data, case-based laboratory-confirmed surveillance data and computer modelling tools (e.g. Measles Strategic Planning [MSP] tool) could be used as well as serosurveys. The MSP tool is an Excel-based modelling tool that takes users through a three-step process: (1) review effectiveness of measles vaccination programme to date, (2) model projected impact of future vaccination strategies, and (3) estimate cost and cost-effectiveness of future strategies. The MSP tool is a cohort model driven by vaccination coverage. It tracks the number of measles vaccinations and estimated infections in each cohort over time to generate a series of tables and graphs and to guide users through data interpretation. An example of using the MSP tool to review population immunity and age groups at risk in Kenya in 2005 was presented. Immunity due to past infection, SIAs, routine MCV1 or maternal antibody could be analysed and the proportion of susceptible population for each age group could be predicted. The use of surveillance data based on reported measles cases, coverage data and vaccination status by age group of measles cases to monitor population immunity in Burkina Faso in 2009 was presented as an example. Serosurveys may be useful (1) if there is any doubt about other ways of measuring population susceptibility, (2) if appropriate protocols, assays and expertise for serosurveys are available, (3) if representative and adequate number of samples from all age groups can be collected, and (4) if the survey can be properly standardized, controlled and analysed. For appropriate serosurveys, sample sizes to represent age, sex and region, type of assay to be used and standardization, analysis and interpretation of data should be carefully considered and planned. PRNT, which is used to
measure neutralizing antibody, is considered as the gold standard, but the ELISA method is also widely used. Standard PRNT protocol for measles was developed by the US Food and Drug Administration (FDA) in 1981. Protective titres of PRNT are considered as >120mIU/ml using international standard 2. Some measles IgG ELISAs have a reasonable correlation with PRNT. Sample sizes should be determined according to expected prevalence of susceptibles, need to make comparisons between subpopulations and precision desired to accurately estimated population immune profile. Approximately 100 samples should be collected from each birth cohort. The age group usually includes individuals up to 30 years of age. He also presented the sample number used by the European SeroEpidemiology Network (ESEN) study as an example. Some measles IgG ELISAs present a reasonable correlation with PRNT. Siemens measles IgG and some other ELISA kits have been compared with PRNT and data have been published. Data show that Siemens assay has relatively good correlation with PRNT, while some other assays have poor correlation with PRNT. Interpretation of Siemens measles IgG assay was presented. Most samples with OD values of <0.1 are PRNT<120mIU/ml, most IgG positive samples have PRNT >120mIU/ml and most IgG equivocals are PRNT>120mIU/ml. He also presented comparison data of three measles IgG ELISA assays (Verion Serion, IBL and in house China CDC assay) with PRNT, which was performed in China CDC. Verion Serion showed higher sensitivity (94.9%) than IBL (58.8%) and in-house assay (84.6%), but specificity was 100% for all three assays. Other IgG assays may be useable for serosurvey if the assays are calibrated with standard sera. ESEN studies used approximately 150 serum panels to calibrate assays used in a study of population immunity in 17 countries. Panel was calibrated with international standard 2 and PRNT, which enabled poorly performing assays to be compared directly with well-performing assays. It was emphasized that assays to be used should be standardized before testing begins. International standards 1 and 2 have been depleted and international standard 3 can be used for calibrating for PRNT. For standardization of serosurveys, PRNT can be used with international standard 3 or secondary standard as controls for each run or ELISA IgG, which has been well calibrated in the past (e.g. Siemens), can be used including a panel of validated control samples. Non-validated assays can be used only after consultation with WHO and with use of comprehensive panel of control samples validated by a reference laboratory. Limitations of serosurveys could include representativeness and limitation (difficult-to-reach populations) of sampling, time, resource and expertise requirement. New techniques for neutralization type assays using fluorochromes are being investigated.

(2) Measles serosurvey in China

Mr Naiying Mao presented a comparison of ELISA assays with PRNT, serosurvey results during 2005–2010, and consecutive serosurveys conducted in some provinces in China. Since hemagglutination inhibition antibody has poor correlation with neutralization antibody, it is not recommended for serosurveys. The China CDC in-house measles IgG assay is a semi-quantitative assay, and commercial kits such as Verion/Serion and IBL are quantitative assays. He presented a cross-section serosurvey conducted in six provinces (Xinjiang, Heilongjiang, Yunnan, Guangdong, Hunan and Anhui) in 2006. This survey used stratified random sampling of 2708 children aged 2–13 years and indirect ELISA for measles IgG (Verion Serion). Equivocal results were considered as non-immune. Results showed that the proportion of population susceptible to measles varied from 11.3% to 3.6%, with a trend of increasing protection level with older age groups. Significant differences in positive rates between different counties were observed. Averaging six provinces, 92.9% of the population was measles IgG positive. In 2010, Hubei province conducted a serosurvey using 700 serum samples collected from people aged 0–25 years from three counties. In two of three counties, children 1–2 years old have not achieved 95% immunity target. Also, 15% of adults over 20 years were susceptible to measles. In Hainan province, a seroprevalence study conducted for both measles and rubella showed 80%–100% positive rates for measles depending on areas. Rubella antibody level was much lower than measles and varied from 52% to 92% depending on areas. In Hebei, immunity levels for measles
were compared before and after SIA in 2010. Overall positive rates before and after the SIA were 95.4% for pre-SIA and 98.9% post-SIA. The results of the serosurvey varied among provinces, prefectures and counties due to different vaccination coverage rates. Measles serosurveys provide important and useful scientific evidence to reflect the level of immunity to measles. Immunity gaps were found among some age groups and in some regions in China. Data analysis should be further strengthened in provinces and collection of samples from hard-to-reach populations would be important to find the immunity gaps in China.

(3) Measles serosurvey in Japan

Dr Katsuhiro Komase presented on the measles serosurvey in Japan and emphasized the situation of having high false-positive rates and low positive predictive values when measles cases are rare. There are other viral rash and fever diseases such as exanthema subitum (caused by HHV6 or 7), erythema infectiosum (caused by parvovirus B19) and hand, foot and mouth disease (caused by enterovirus 1, coxsackievirus A10, coxsackievirus A16). There are some IgM cross-reactions among parvovirus B19, HHV-6 and measles. In general, Denka kit is more sensitive but less specific than Siemens kit. Therefore, since the false-positive rate is higher with Denka kit, Japan could be overestimating measles cases. Most commercial laboratories use IgM ELISA (10 000–20 000 samples per year), while prefectural institutes of public health use RT-PCR for the laboratory confirmation of measles cases. In 2009, about 13 000 samples were tested for measles IgM using Denka kit and about 600 cases were positive and 325 were equivocal for measles IgM. Double-checking of Denka measles IgM positive cases using another kit or other methods (e.g. PCR) may be useful to reduce the false-positive rate. An evaluation committee for measles diagnosis may be necessary during the measles elimination stage in Japan.

2.3.10 Data management and reporting measles and rubella laboratory data

(1) Monthly laboratory data reporting

Mr Benjamin Bayutas presented the status of measles/rubella laboratory data reporting from countries. Currently, 16 laboratories send monthly data to the Regional Office. Eight laboratories use an Access database format, while the other eight laboratories use Excel spreadsheets. Advantages of a standard reporting format such as automatic data checks, reliable data transfer, automatic report generation and data archiving were presented. Additional reports on virus isolation and RT-PCR were added to the new laboratory database reporting format. The way to use the updated measles/rubella laboratory database was demonstrated. Coordination with laboratories with customized databases is onward from October 2011. An updated version will be released to laboratories by the end of September 2011.

(2) WHO and Measles Nucleotide Sequence (MeaNS) Database

Mr David Featherstone presented the details of WHO genotype and MeaNS databases. Timeliness of virus detection and genotyping is a criteria of WHO laboratory accreditation. Results of virus detection and genotyping should be completed within 2 months of receipt of specimens, with data reported to WHO monthly, for at least 80% of samples for genetic analysis. However, in reality, molecular information should be shared as soon as possible. He explained three different ways of sharing measles sequence or genotype data: (1) WHO genotype database, which was established in 2006 for sharing genotype and epi data with restricted access (only for laboratory network); (2) MeaNS sequence database, which was established in 2008 for sharing sequence and epi data with automatic reporting to WHO and restricted access; and (3) GenBank, which is a sequence database that is open to the public. LabNet members need to report to a WHO databases and preferably to MeaNS. As of September 2011, 10 556 measles virus strains from 131 countries and 990 rubella virus strains from 44 countries have been deposited. Twenty-
three genotypes and one provisional genotype were reported for measles, and nine genotypes and four provisional genotypes were reported for rubella. The MeaNS database, which is operated by the Health Protection Agency (HPA), United Kingdom receives the sequences of 450bp N gene of measles virus. In 2010, about 1400 sequences from 70 countries were reported, and in 2011 (as of September), about 1200 strains from 48 countries were reported. Among Western Pacific Region countries, China represents about 70% of regional data for measles genotypes, with the rest of the cases coming from Australia, Japan, Malaysia and Singapore. In 2010, about 50% of sequencing data were reported within 2 months of onset of cases, and 80% were reported within 3 months. He also showed global distribution of measles genotypes. While D4 strains are widely circulating in European countries, B3 and B2 strains circulated in African countries in 2010. In the Western Pacific Region, H1 and D9 genotype strains were two major strains that circulated. He also indicated the countries with laboratory-confirmed cases without genotype information in 2010. Only limited information is available for rubella genotypes. In 2010, only 13 countries reported genotype information, while 108 countries reported laboratory-confirmed rubella cases. In 2011, D4 strains continued to circulate in European countries and B3 strains in African countries. In 2011, H1 strains were mainly detected in China, and other countries in the Western Pacific Region mainly detected D9, D4 and D8 strains.

The MeaNS sequence database (www.who-measles.org) was established for depositing measles sequences files, genotyping and comparison/phylogeny with measles viruses from other countries. Additionally, data can be automatically submitted to the WHO genotype database and, if agreed, to GenBank. The MeaNS database can provide measles news and articles, some of which can be obtained without registering. It is planned to add mapping features in the future. LabNet capacity for molecular surveillance was improved in the past six years in terms of the number of submissions and timeliness of reporting. Close monitoring of molecular surveillance data is required to determine progress towards measles elimination. However, surveillance gaps among countries still exist and some outbreaks are being missed. In 2010 and 2011, 13 and 10 countries submitted rubella genotype information to WHO, respectively. Most rubella genotype information in 2011 was received from China. To register with the WHO database and MeaNS database, each individual using the databases should register. Institutional access is not recommended.

2.3.11 Laboratory diagnosis of mumps outbreaks in 2011

(1) Mumps outbreak in 2011: Lao People’s Democratic Republic and Mongolia

Dr Janice Lo from Hong Kong (China) RRL presented the laboratory diagnosis of mumps cases from outbreaks in the Lao People’s Democratic Republic and Mongolia in 2011 as well as mumps cases in Hong Kong (China). Testing methods to diagnose mumps cases include IgM ELISA using Microimmune kit, virus isolation (LLC MK2 cells) with IFA and RT-PCR, real-time PCR and sequencing of 316bp of SH gene and complement fixation test using paired serum samples. Respiratory specimens, saliva, buccal swab and urine samples can be used for virus isolation and molecular detection. When mumps cases are confirmed by Microimmune ELISA with serum samples, the laboratory performs confirmatory ELISA using Siemens ELISA. When negative results are obtained, the laboratory asks for a second batch of serum samples for complement fixation and another IgM test. Additional saliva and buccal swab samples are also requested for PCR and virus isolation. Real-time PCR is conducted for non-serum samples, and, if positive, nested genotyping RT-PCR is conducted followed by sequencing. In 2009 and 2010, 220 and 153 samples from Hong Kong (China) were tested for mumps and 8 and 5 were laboratory-confirmed, respectively. In 2011 (as of July), 62 samples were tested and 2 cases were laboratory confirmed. In 2008, H genotype strain was detected, and in 2009, one B, three F and two G genotype strains were detected. In 2010 and 2011, F genotype strains were detected. Sensitivities of different diagnostic methods were compared using different samples with 13
laboratory-confirmed mumps cases. Results showed that 60% were positive with ELISA IgM assay using 15 serum samples, 11% were positive with culture, 77.8% were positive with RT-PCR using 9 saliva samples, 100% were positive with RT-PCR using 4 throat swab samples (no culture positive), 33.3% were positive with culture, 100% were positive with RT-PCR using 3 nasopharyngeal swab samples and 50% were positive with RT-PCR (no culture positive) using 2 urine samples. For mumps IgG testing, Human kit is first used and when the result is negative, VIDAS kit is used in this laboratory. The results of confirmation tests of samples from outbreaks in the Lao People’s Democratic Republic and Mongolia in 2011 were also presented. Concordance rates of mumps IgM tests of serum samples from the Lao People’s Democratic Republic (n=6) and from Mongolia (n=18) were 100% and 78%, respectively. Ten throat swab samples from the Lao People’s Democratic Republic and 15 throat swabs and 18 urine samples from Mongolia were negative for virus isolation. From three throat swab samples from the Lao People’s Democratic Republic, enteroviruses (coxsackievirus A16 and rhinovirus) were isolated. Four out of 10 throat swab samples from the Lao People’s Democratic Republic were positive by RT-PCR and G genotype strains were detected. Eleven out of 13 throat swab samples and 5 out of 15 urine samples from Mongolia were positive by RT-PCR and F genotype strains were detected. Challenges for mumps could be low positive IgM rates among the population with high immunization coverage, insufficient serum/follow-up samples for confirmatory testing, inadequate non-serum samples for virus isolation and PCR/genotyping and inadequate clinical/epidemiological information for test information and further testing.

(2) Laboratory surveillance on mumps in China

Dr Aili Cui presented the mumps epidemiology, laboratory diagnostic methods and molecular surveillance of mumps virus from 1995 to 2011 in China. Mumps virus was first identified as a new mumps genotype in 1995. Mumps surveillance was incorporated into the National Diseases Reporting Information System in 2004, and mumps vaccine was introduced into the national immunization programme in 2007. Mumps incidence in China from 2004 to 2010 ranged from 17 to 22 per 100 000 population. In 2010 and 2011, 297 196 and 283 663 mumps cases were reported, respectively. The epidemiological data showed the incidence of mumps was very high during 2004–2011, with the average incidence of 21 per 100 000 in China. Most mumps cases occur among children aged 5–15 years old. The epidemic peak occurred every three years, with the predominant peak in April–July and small peaks in November–December. Serological methods using hemagglutination inhibition, complement fixation, neutralization test and ELISA, virus isolation using Vero or Vero/Slam cells and molecular methods using RT-PCR targeting SH gene (316bp) or real-time PCR targeting Hemagglutinin gene (64bp) are used for the laboratory diagnosis of mumps. The 316 bp region of the SH gene, which is widely used among WHO network laboratories, has high nucleotide variation. Mumps ELISA using CSF samples can confirm mumps encephalitis or meningitis. Provincial laboratories in China perform virus isolation and real-time PCR using virus isolation, samples (throat swab, saliva, CSF and urine). Once positive for virus isolation or real-time PCR, RT-PCR followed by sequencing is conducted. Virus isolates are sent to China CDC for further analysis by real-time PCR, RT-PCR and sequencing. During 1995–2011, 16 provinces conducted mumps virological surveillance. Between 2001 and August 2011, 260 mumps virus isolates were obtained from 16 provinces and 73 strains were genotyped. Genotype F was the predominant genotype circulated in China from 1995 to 2011, and the genetic diversity of genotype F strains in China was 0%–7.0%. Multiple transmission chains occurred in different provinces or years. However, similar mumps viruses also circulated in different provinces or years. To improve the sensitivity of case diagnosis and genotyping, the real-time RT-PCR method has been introduced into China’s measles laboratory network.
Dr. Paul Rota presented laboratory diagnosis of mumps in the United States of America. RT-PCR and cell culture are the best diagnostic tests currently available to detect mumps infection in both unvaccinated and previously vaccinated individuals. Serological tests for mumps IgM are widely available but often fail to detect IgM in serum samples from previously vaccinated persons. There are some challenges with mumps diagnosis: persons with a history of mumps vaccination may not have detectable mumps IgM antibody regardless of the timing of specimen collection. The ability to detect IgM varies by vaccination status and is highest in unvaccinated persons (80%–100%), intermediate in one-dose vaccine recipients (50%–80%), and lowest in two-dose vaccine recipients (14%–50%). Absence of a mumps IgM response in a vaccinated or previously infected individual presenting with clinically compatible mumps does not rule out mumps as a diagnosis. Standard serologic assays that detect mumps IgM are commercially available in various EIA. A capture IgM EIA (non-quantitative) that incorporates a recombinant mumps nucleocapsid protein as the antigen is used at US CDC to detect mumps IgM.

In 2009, a mumps outbreak occurred in a northern region of the country. From the outbreak, 297 samples were analysed. The results of mumps IgM detection using the US CDC capture IgM assay and two commercially available kits were compared using virus isolation as a method of gold standard. Gaps of positive ratios were found. The positive ratios were 52% for CDC IgM capture assay, 12.5% for commercial indirect EIA and 29% for commercial IgM capture assay. For mumps IgG detection, a commercial, indirect EIA (non-quantitative) is used for detection of IgG but the presence of IgG is not a measure of immunity. No serological test available for mumps consistently and reliably predicts immunity. A single serum sample tested for mumps-specific IgG is not useful for diagnosing acute mumps infections. The presence of mumps-specific IgG indicates that an individual has experienced mumps vaccine or mumps virus infection. Paired serum samples from vaccinated persons, even if appropriately timed, may not show a rise in IgG titer.

Mumps virus has one serotype and 12 recognized genotypes. Genotyping is based on sequence of the SH gene, and real-time PCR targets the N gene. The molecular epidemiology of mumps is not as well established as for measles or rubella. Obtaining sequence information from representative strains of mumps will help to build a global database. During the period of 2006–2010, genotypes G (93%, n=351), H (3%, n=13), C (1%, n=4), A (1%, n=3) and K/M (1%, n=5) were detected in the United States. Genotype G mumps strains are also frequently detected in Europe. The primers targeting the N gene for real-time PCR increased sensitivity of viral detection compared to the protocol using SH gene. Among 124 buccal swabs collected from a mumps outbreak in 2009, real-time PCR targeting the N gene was able to detect 83% compared to 53% using the SH gene. Laboratory testing should be used to confirm suspected outbreaks of mumps, not to confirm every suspected case, and should be only conducted on suspected cases meeting a stringent case definition. Because of the limited utility of serologic testing, laboratories with capacity for PCR should consider establishing the mumps rRT-PCR assay as a diagnostic method. The laboratory is encouraged to establish a genetic baseline for wild type mumps viruses.

2.3.12 Discussion on current issues

(1) Use of real-time serum samples for genotyping in Hong Kong (China)

Dr. Janice Lo presented the experience of using confirmatory serum samples for genotyping. Nested PCR using the primers designed by HPA is used for detecting measles virus from serum samples. HPA primers cover only 445 bp or 444bp of the WHO-recommended genotyping window of 450bp (1234–1683). The importance of selecting suitable sample
dilutions for molecular detection was explained to have reproducible and consistent results. The dilution selected should not be too low as the results may fluctuate above and below the cut-off due to normal variations. It should also not be too high because it will be of limited use for borderline monitoring and may not identify subtle changes. Therefore, the optimal sample dilution should be 10-fold above the limit of detection.

Table 4. Measles RT-PCR results, Hong Kong (China), 2006–2011

<table>
<thead>
<tr>
<th>Days after rash onset</th>
<th>Serum</th>
<th>NPA</th>
<th>TS/TNS</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>RT-PCR</td>
<td>Culture</td>
<td>RT-PCR</td>
</tr>
<tr>
<td>&lt;0-3</td>
<td>91.8%</td>
<td>81.8%</td>
<td>83.3%</td>
<td>93.8%</td>
</tr>
<tr>
<td>4-7</td>
<td>98.5%</td>
<td>78.2%</td>
<td>75.0%</td>
<td>100%</td>
</tr>
<tr>
<td>&gt;7</td>
<td>100%</td>
<td>50.0%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Overall</td>
<td>95.4%</td>
<td>77.0%</td>
<td>80.9%</td>
<td>95.7%</td>
</tr>
</tbody>
</table>

^until 30th Jun 2011

Serum samples, nasopharyngeal aspirate, throat swabs and urine samples that were collected on different dates after rash onset were used for RT-PCR and viral culture or IgM detection (serum). Detection rates of different samples collected on different days were compared (Table 4). Serum samples collected 0–3 days after rash onset showed 81.8% positive rates using RT-PCR compared to 91.8% using IgM detection. Serum samples collected 4–7 days after rash onset showed 78.2% positive rates using RT-PCR compared to 98.5% using IgM detection. For samples collected after 7 days, RT-PCR detection rate was 50% compared to 100% for IgM detection. Overall, the RT-PCR positive rate was 77% compared to the ELISA IgM positive rate of 95.4%. For throat or nasal swab samples and urine samples, RT-PCR detection rate was higher (95.7%) than culture positive rates of 80.9% when collected within 7 days after symptom onset. Rubella genotyping using serum samples is more difficult due to the amplification of longer fragments and the method needs further optimization. Overall, the RT-PCR positive rate using serum samples was 39.6% for rubella compared to 77% for measles. With virus isolation samples, the RT-PCR detection rate for rubella was much lower than that for measles. Serum samples can be still useful for measles genotyping when viral isolates are not available, and best results can be obtained when the serum samples are collected within 7 days after rash onset. Clinical and epidemiological information should be obtained to develop baseline data for devising testing strategy.

(2) Use of real-time and conventional RT-PCR for case classification and molecular surveillance of measles virus

Dr Paul Rota presented the use of real-time and conventional RT-PCR for case classification and molecular surveillance of measles virus. He emphasized that nested PCR is recommended only for experienced global or regional laboratories. The TaqMan real-time PCR was discussed since its use can significantly increase the specificity of the detection. Real-time
assays are more sensitive than conventional PCR, can detect 10–100 copies of viral RNA/sample in a high throughput format and can produce results in 3–4 hours. It can help to confirm a case when serologic results are inconclusive. However, real-time PCR negative results do not rule out a case, and sequence information from the conventional PCR product is required for genotype and confirmation of vaccine reactions. The real-time PCR product cannot be used for molecular epidemiologic studies. For real-time RT-PCR assays, Ct values of RNA standards can be used to generate a standard curve, and the standard curve is used to calculate the number of copies of measles genome in the samples. For measles diagnosis, all samples with a positive signal (Ct<40) are reported as positive irrespective of copy numbers. The purpose of the standard curve in the measles assay is to ensure that the assay had sufficient sensitivity to detect samples with low copy number and to ensure that the assay is valid. To ensure that the quantity and quality of the RNA extracted from the specimen are sufficient for real-time analysis, rRT-PCR for RNase P is performed. Quantitative PCR human reference total RNA is used as a positive control for the RNase P rRT-PCR. Specimens are tested in duplicate or triplicate. A total of 270 measles samples from suspected measles cases in USA during January–June 2011 were tested by rRT-PCR. The results showed 117 (43%) positive, 130 (48%) negative and 23 (9%) indeterminate. Synthetic RNA (MeVN-3in) should be used to prepare positive control serial dilutions for measles virus real-time PCR (included in genotyping kit version 2). A new measles genotyping (conventional) RT-PCR kit with increased sensitivity has been developed with improved primers of MeV214 and MeV216 to detect 634bp N terminus of the N gene. Previously recommended primer pair MV60/MV63.3 required 100 times more input RNA though it amplifies all 11 genotypes tested. A new control synthetic RNA (MeV-N3in) was also included to reduce the risk of misinterpretation due to cross-contamination. The synthetic RNA can be used with the primers in genotyping kit version 2 as well as with the previously recommended primers MV60/MV63.3. RNA control can also be used for genotyping RT-PCR or real-time diagnostic RT-PCR. Measles RNA or RT-PCR can be extracted from either infected cells or clinical samples.

The countries and regions at or near the elimination phase are faced with challenges for measles classification and diagnosis because (1) outbreaks often include cases of primary and secondary vaccine failure, (2) vaccine reactions are detected and can be confused with disease, and lastly, (3) collection of appropriate samples for virologic surveillance is difficult. Modified measles cases due to secondary vaccine failure can be presented with milder symptoms such as reduced fever, modified rash/shorter duration and without one of the three Cs (cough, coryza and conjunctivitis). Modified cases are identified in conjunction with outbreaks of classic measles due to heightened surveillance during measles outbreaks. Modified cases due to secondary vaccine failure can be presented with positive or negative measles IgM result and positive IgG with high avidity. Cases can be confirmed by PCR (Table 5).
Table 5. Utility of new assays for the measles diagnostic toolbox

<table>
<thead>
<tr>
<th>Classification</th>
<th>Clinical</th>
<th>IgM</th>
<th>IgG</th>
<th>Avidity⁴</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine reaction</td>
<td>Fever/Rash</td>
<td>P</td>
<td>P or N</td>
<td>Low</td>
<td>Positive and Sequence indicates vaccine strain</td>
</tr>
<tr>
<td>Vaccine reaction</td>
<td>Fever/Rash</td>
<td>N</td>
<td>P</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Measles</td>
<td>Meets CCD*</td>
<td>P</td>
<td>P or N</td>
<td>Low can confirm IgM</td>
<td>Positive PCR can confirm IgM</td>
</tr>
<tr>
<td>Measles (but recently vaccinated)</td>
<td>Modified⁹</td>
<td>P</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles-Primary Vaccine Failure</td>
<td>Meets CCD*</td>
<td>P</td>
<td>P or N</td>
<td></td>
<td>Positive PCR can confirm case</td>
</tr>
<tr>
<td>Secondary vaccine Failure</td>
<td>Modified⁹</td>
<td>P or N</td>
<td>P</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴Clinical case definition (CCD)  
⁹Modified or mild presentation does not meet CCD  
⁻Collection of sample is ≤ 3 weeks after rash onset

(3) Shipping samples on filter paper

Dr Paul Rota presented on shipping viral isolates and serum samples on filter paper (#903). Filter papers can be used to submit viral isolates and serum samples for sequence analysis and genotyping and to ship serum samples (spotted directly on to filter) for confirmatory testing. Because both measles and rubella viruses have been recovered from standard specimen paper (#903), samples on this type of paper should be treated as infectious materials. Therefore, the specimen paper needs to be shipped using the triple-layer containment method for Category B infectious substances (UN 3373) and can be shipped at room temperature. Shipment of PT panel samples and control RNA for RT-PCR can be shipped using this type of filter paper. Another type of filter paper that can be used for PCR is the Whatman FTA Elute Micro Card (CAT # WB120410 for 100 with 4 circles (11 mm), 120401 for 25 with 4 circles, 120411 for 25 with 1 circle and 120412 for 100 with 1 circle), which has been chemically treated to lyse cells and denature proteins on contact (cards have an expiry date). Therefore, samples on this card are not infectious and can be stored and shipped at room temperature. One sample is used per card and RNA can be extracted using Qiagen viral RNA mini kit. This card should not be used for shipping serum samples because antibody molecules will be denatured. To prepare FTA cards, 40ul of clarified vial lysate is added to one circle on the FTA card in a biosafety cabinet. The isolate should be added in a concentric pattern to avoid overloading the chemicals on the card. This card should then be allowed to air-dry for 1–3 hours in a biosafety cabinet before being placed in an airtight Ziploc bag with desiccant packs to dry for three days at room temperature. Cards with vial lysate are stable at room temperature for up to two months, but it is recommended to store them at 4° C. Cards can be prepared a few days before shipping. For extraction of RNA from FTA discs using QiaAmp viral RNA mini kit, a clean 6 mm punch or clean disposable scalpel can be used for each sample. Extreme care should be taken to prevent cross-contamination. It is important to thoroughly decontaminate the hole punch between samples using bleach. RNA can be extracted with Qiagen RNA extraction kit. Stability testing of the samples on filters showed that measles RNA is stable for at least one month on FTA cards. On the other hand, rubella RNA on FTA cards and #903 cards is stable for at least six months – one year for diagnostic RT-PCR and at least one month for genotyping fragments. FTA Micro Card (Cat # WB120210) is used in WHO polio laboratories. Complete protocols are available from US CDC on request. The next steps are to develop a standard protocol for transporting...
samples on filter papers for the Western Pacific laboratory network (FTA, heat inactivation), to perform field tests of the protocol and to explore the use of FTA cards for transporting clinical samples for molecular epidemiology.

3. CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

The Third Meeting on Vaccine-Preventable Diseases Laboratory Networks in the Western Pacific Region was held in Manila, Philippines from 5 to 9 September 2011. The meeting was organized in three parts: (1) polio laboratory network, (2) biosafety training, and (3) measles and rubella laboratory network. Participants included about 60 representatives from polio and measles/rubella laboratories from this Region and advisers from US CDC and WHO Headquarters.

A one-and-a-half day session of the polio laboratory network was held from 5 to 6 September to review the performances of the poliomyelitis network, identify challenges of network laboratories and ways to strengthen the quality of the performances, and discuss the implementation of the recommendations from the network’s meeting in February 2010.

3.1.1 Polio Laboratory Network

The performance of the regional polio laboratory network is sustained at polio-free certification standard, and AFP surveillance activities are efficiently supported by the polio laboratory network. The network laboratories provided critical evidence in support of the continued polio-free status of the Region. All network laboratories except China implemented the new algorithm for virus isolation. For 2010 virus isolation PT samples, all network laboratories except China used the new algorithm for the first time. All polio laboratories obtained a 100% score, and all seven ITD laboratories passed ITD proficiency testing with a 100% score and were accredited for ITD function.

After the introduction of real-time PCR for ITD in this Region, all seven ITD laboratories began participating in proficiency testing that was prepared by US CDC. While five laboratories are using real-time PCR, two laboratories (Hong Kong [China] and New Zealand) are still using the conventional PCR ITD. The ITD PT samples that were distributed to the seven ITD laboratories will also be used for sequencing PT.

Being a polio-free region for more than 10 years, network laboratories in the Western Pacific Region have been actively involved in supplementary enterovirus or environmental surveillance. In particular, China established a very extensive hand, foot and mouth disease laboratory network based on existing polio and measles/rubella laboratories. Some countries in the Region (e.g. Australia, China, Japan, Malaysia and the Philippines) are either conducting or are interested in environmental surveillance to supplement AFP or enterovirus surveillance. Network laboratories are involved in those activities.

Since the change of definition of type 2 VDPV during the global polio laboratory network meeting in 2010, China has detected more type 2 VDPVs. The timeliness of sharing the laboratory results of VDPV detection with WHO has been improved in 2010–2011.
China expanded environmental surveillance in several provinces after organizing the third workshop in August 2010. Australia also introduced environmental surveillance, and Malaysia is planning to conduct environmental surveillance this year. The network continued to discuss the role further during the sessions on detection of polioviruses from non-AFP cases and on sharing experience of polio network laboratories for the laboratory diagnosis of hand, foot and mouth disease.

The establishment of environmental and enterovirus surveillance in a number of countries and areas in the Region has provided valuable data to support the maintenance of polio-free status of the Region. These virological surveillance systems can supplement AFP surveillance.

On 26 August, China CDC confirmed four wild poliovirus type 1 strains, which are closely related to viruses circulating in Pakistan, through collaboration with the WHO global specialized laboratory in US CDC and the Pakistan NIH laboratory. This event clearly shows the important role of the polio laboratory network and how quickly it can provide critical information to the national programmes and WHO. All polio network laboratories in this Region should be prepared to detect polioviruses with the highest efficiency in case of importation.

3.1.2 Biosafety training

Laboratory safety is of utmost importance to GPLN. The biosafety training session, which used six modules developed by GPLN, was held for one and a half days during this meeting. Meeting participants from both the polio and measles/rubella sessions joined the training and were divided into three groups. Trainers and rapporteurs were selected from the network laboratories. Each group had trainers for each module and two rapporteurs/presenters. The biosafety training was designed to share important messages and good practices, stimulate discussion and promote class participation, allow participants an opportunity to share opinions and experiences on working safely in the laboratory, and identify areas for improvement so that work can be performed more safely and the risk of release of poliovirus or other infectious pathogens to the environment can be minimized. At the end of the training, rapporteurs from each group presented a summary of discussions during the training.

3.1.3 Measles and Rubella Laboratory Network

It was concluded that measles and rubella network laboratories provide high-quality support to achieve the regional goal of measles elimination by 2012 by confirming suspected cases and identifying measles virus genotypes circulating in the Region.

The network consists of one global specialized laboratory in Japan, three regional reference laboratories in Australia, China and Hong Kong (China), 13 fully functional national measles/rubella laboratories and 31 provincial and 331 prefectural laboratories in China. Among the 48 laboratories that are accredited by WHO, 47 laboratories were fully accredited as of August 2011. All provincial laboratories in China, including the Tibet laboratory that was assessed in August 2010, have been reviewed and accredited. Accreditation for prefectural laboratories in China is conducted by provincial laboratories in collaboration with China CDC. To ensure the quality of testing in national laboratories, confirmatory testing has been conducted in WHO regional laboratories. All network national laboratories in the Region have sent a proportion of serum samples to WHO RRLs. The concordance rates of IgM testing for measles and rubella were >90%.

In 2010, network laboratories including those in China tested about 60,000 samples for measles IgM (16,880 samples were tested in non-China countries). Among samples tested, 30,555 samples (including 4,703 samples from non-China countries) were positive for measles.
total of 38,489 samples were tested for rubella (14,823 samples were tested in non-China countries). Among samples tested for rubella, 6,749 samples (including 3,920 samples from non-China countries) were positive for rubella IgM. In 2010, NMLs in the Philippines and Vietnam tested a large number of samples from measles outbreaks. In 2011, Cambodia, Malaysia, and the Philippines detected many measles positive cases from outbreaks. Cambodia and Vietnam also reported large proportions of rubella-positive cases in 2011.

In 2011 (January–June), in non-China network laboratories, 13,756 samples were tested for measles IgM and 4,126 samples were positive, while 11,052 samples were tested for rubella IgM and 4,100 samples were positive. In 2011, China began reporting some surveillance and laboratory data. From January to June 2011, 22,843 samples were tested among China network laboratories and about 7,000 samples were positive for measles IgM.

As the role of the measles and rubella laboratory network also extends to molecular surveillance, laboratories with virus isolation, molecular diagnosis and sequencing capabilities are encouraged to perform virus isolation, sequencing and genotyping. Genotype and sequencing information are submitted to the WHO genotype and MeaNS database by national or regional laboratories. Genotype data on recent measles virus strains are available from all countries except Pacific island countries. In 2010–2011, Hong Kong (China) RRL continued to provide excellent support to identify genotypes of measles viruses circulating in Cambodia, the Lao People’s Democratic Republic, Macao (China), Malaysia, Mongolia, the Philippines, and Vietnam using confirmatory serum or virus isolation samples.

Laboratories with the capacity to conduct virus isolation and molecular detection were further trained during the follow-up training in November 2010 in Hong Kong (China). After the training, more network laboratories have become involved in virus isolation and molecular detection of measles and rubella viruses.

As of August 2011, 15 out of 17 laboratories that are supposed to send monthly laboratory data to the WHO Regional Office have been sharing laboratory data on a monthly basis. Laboratory data including measles genotypes identified from each country have been included in the Measles and Rubella Bulletin of the WHO Regional Office from 2011.

The network should continue to make full efforts to obtain genotype and sequence information on measles and rubella viruses circulating in the region. The laboratories should regularly communicate and collaborate with the national surveillance or epidemiology groups and the WHO Regional Office to minimize discrepancy of laboratory and surveillance data, and the delay in testing of samples and regular reporting of laboratory data to the WHO Regional Office.

3.2 Recommendations

3.2.1 Polio session recommendations

(1) Implementation of new test algorithm: With full implementation of new test algorithm in the Region (except China), network laboratories should closely monitor the timeliness of reporting: virus isolation results within 14 days and ITD results within 7 days. Network laboratories without ITD capacity should refer L20B positive isolates to designated RRLs for characterization within 7 days of detection. Remaining subnational polio laboratories in China should also consider using the new algorithm and sending L20B isolates to China CDC as soon as possible to reduce the time to identify polioviruses.
(2) Reporting wild polioviruses and VDPV detections: To reduce the risk of in-country and international spread of wild polioviruses or VDPVs and the risk of outbreaks of poliomyelitis, laboratories should promptly report wild polioviruses or VDPV detections to national authorities, especially country EPI units, who should promptly share such information with WHO country and regional offices within 24 hours.

(3) Cell sensitivity testing results: As recommended during the previous laboratory network meetings in 2008 and 2010, all network laboratories should report results of cell sensitivity tests to the WHO laboratory coordinators for review within 48 hours of completing each test run to facilitate collaboration in rapidly identifying problems and implementing actions to optimize procedures for accurate detection of polioviruses, including re-testing of specimens, as appropriate. Results should be provided in a trend chart including full details of titration experiments.

(4) 2011 ITD and sequencing PT: Laboratories that are participating in ITD PT and have sequencing capacity are encouraged to test those ITD PT samples for sequencing. ITD results should be reported within 7 days after receipt of samples in the laboratory, and sequencing results should be reported within 2 weeks after ITD result. Primers for sequencing can be obtained from US CDC. The WHO Regional Office needs to facilitate this process.

(5) Accreditation of polio sequencing laboratories: All ITD laboratories with sequencing capacity should also participate in relevant quality assurance and proficiency test programmes to be accredited as a polio sequencing laboratory. Laboratories accredited for sequencing can provide nucleotide sequence data for programme use.

(6) Expansion of the real-time RT-PCR assays to other network laboratories can be considered for the laboratories with existing real-time PCR equipment, infrastructure and experience with real-time PCR assays.

(7) Shipping of isolates: All polio laboratories should work closely with the Ministry of Health to develop documented procedures to reduce the time to ship samples to the regional or global laboratories for further analysis.

(8) Data management: Network laboratories are recommended to use the new standardized polio laboratory database once it becomes available.

(9) Reporting non-AFP data to national authorities and WHO: Network laboratories should report results for polioviruses isolated from all sources including those identified through special surveys such as environmental surveillance and enterovirus surveillance. Reports for non-AFP specimens should be submitted on a monthly basis and polioviruses isolated from non-AFP cases can be reported using the WHO Headquarters format until the standardized reporting format of AFP cases is distributed. Detections of wild polioviruses or VDPVs from non-AFP sources should be reported immediately to national authorities and WHO.

(10) Timeliness of reporting non-AFP data: Recognizing the importance of maintaining the polio-free status of the Region, poliovirus isolates from all specimen sources should be rapidly and appropriately characterized to identify isolates that can potentially circulate. Poliovirus isolates and L20B positive specimens from non-AFP sources should be referred for ITD testing using WHO-recommended procedures within 7 days of detection.

(11) Reporting of polioviruses from patients >= 15 years old: Polioviruses isolated from patients >=15 years of age who present with clinical symptoms compatible with poliomyelitis or...
AFP should be reported to the national authority and WHO in a timely manner. Laboratories can use the same reporting format as for AFP data.

(12) Supplementary surveillance: Considering the significant value of enterovirus and environmental surveillance, countries with the capacity are encouraged to conduct enterovirus and environmental surveillance to supplement AFP surveillance. The frequency of environmental sampling may be increased depending on seasonal trend, or special epidemiological situations. Countries currently conducting environmental surveillance are requested to share their protocols with WHO for reference.

3.2.2 Biosafety training session recommendations

(1) NPLs should communicate with the Ministry of Health or other appropriate national biosafety authorities regularly to address relevant biosafety issues in their laboratories.

(2) The head of NPLs should liaise with the management of the institute and/or designated biosafety officer to nominate a biosafety focal point and conduct biosafety training of personnel using the training materials developed by GPLN to strengthen biosafety and biorisk management in individual laboratories.

(3) Each NPL will also be required to nominate a biosafety focal point who will liaise with WHO.

(4) Training participants should conduct at least one biosafety training workshop within the institute (mandatory for NPL and recommended for other sections of the institute) within six months, provide feedback for the training modules and share the outcomes of the training with the WHO Regional Office.

(5) Training materials (videos) should be made available to all NPL staff, and other staff of the institute if necessary, for the induction of new staff and refresher training.

3.2.3 Measles and rubella session recommendations

(1) Data reporting

Laboratory data should be reported to the WHO Regional Office on a monthly basis by the 10th day of the month. A report should be sent to the laboratory coordinator at the WHO Regional Office (jeey@wpro.who.int) and data management team (wpr_epidata@wpro.who.int). The laboratory data reporting format that has been revised using MS Access should be fully adopted by network laboratories using DBS and performing virus isolation/molecular detection. Countries where case-based reporting is not feasible (e.g. China) should make every effort to share aggregate data with the WHO Regional Office in a format and frequency agreed upon with the regional office.

(2) Confirmatory testing

The confirmatory testing mechanism of serum samples established in the Region should be maintained. It is recommended that national laboratories send a representative 10% of samples or a minimum of 15 samples to the designated RRL or GSL, at least annually but preferably twice a year. A table including a list of the samples and the raw data (OD readings) obtained by the national laboratory should be included with the shipment and a copy e-mailed to the regional or global specialized laboratory. Before sending samples, the national laboratories should notify and
consult with the regional laboratory coordinator to confirm the number and selection of samples to be sent.

(3) Molecular surveillance

(a) Laboratories are encouraged to submit representative genotype/sequence information on their measles and rubella viruses to the WHO genotype and MeaNS database (for measles), preferably on a “real-time” basis, but at least by the end of the month in which the genotyping was completed. A copy or summary copy of the information should be sent also to the regional laboratory coordinator.

(b) Laboratories that participated in the regional hands-on laboratory training in 2009 and 2010 are encouraged to perform virus isolation and/or molecular detection of measles and rubella viruses to identify genotype and obtain sequence information on circulating measles and rubella viruses.

(c) The laboratory network should utilize the well validated tools and samples available for enhancing molecular surveillance where appropriate, such as oral fluid (ORACAL kit), throat swabs and urine, as samples for virus detection using validated and standardized PCR methods including the use of a validated, appropriate PCR controls. The Region should work towards validated protocols.

(d) The laboratories that will roll out the ORACAL kits to enhance molecular surveillance are requested to report the results during the next regional VPD laboratory network meeting.

(e) Some laboratories may be selected to pilot-test the use of FTA cards for submitting viral isolates to the RRL or GSL for genetic testing.

(4) Use of dried blood spots

(a) Dried blood spots are in routine use in some countries (e.g. Cambodia). Consideration should be given to introducing the DBS specimen collection technique in countries or areas where there are challenges in collecting serum/blood samples and/or shipping under cold chain to national or reference laboratories.

(b) The IgM results using DBS as an alternative sampling method should be monitored closely for any significant discrepancy of results with serum samples. Laboratory testing records should clearly note inadequate samples.

(c) The reagents needed for processing DBS samples (e.g. skimmed milk powder) need to be supplied without interruption.

(5) Reporting of WHO measles and rubella PT results

(a) All network laboratories should report 2011 PT results within two weeks or 14 working days to VIDRL and the regional coordinator using the agreed reporting format after the arrival of samples in the laboratory. All 31 provincial laboratories in China that participate in the WHO global PT should also use the same format and include OD values.

(b) Non-network laboratories that will receive WHO PT samples from this meeting should also report the PT results within two weeks using the same format.

(6) Involvement of molecular detection work and PT
Network laboratories with the capacity to conduct molecular detection of measles and rubella should participate in molecular PT from 2012. A regional protocol to perform molecular detection for measles and rubella and molecular PT will be developed by the end of 2011.

(7) Measles and rubella positive samples for quality control and global PT

All network laboratories should store measles and rubella positive serum samples at temperatures that are -20°C or lower to use for internal laboratory controls, for global proficiency test panels and for virus identification. Those laboratories with stocks of positive samples (preferably volumes larger than 0.5ml) are requested to contact the regional laboratory coordinator to facilitate using these samples in the WHO proficiency and quality control programme.

(8) Designation of subnational laboratories

In countries with difficulties of shipping samples to NML in a timely manner, designation of additional subnational laboratories can be considered. The NML should be responsible for ensuring the quality of testing conducted in subnational laboratories by regularly re-testing a proportion of samples from subnational laboratories.

(9) Quality assurance of non-NML laboratories and commercial laboratories

In countries where most measles and rubella IgM testing is performed in non-NML laboratories or private/commercial laboratories, it is vital that the performance of those laboratories be monitored. Performance of these laboratories may be assessed through an external quality assurance programme, and pre-existing quality assurance data should be assessed by the national laboratories, where possible. The results of those assessments should be submitted to WHO. In countries with low incidence of measles and rubella, NMLs should consider confirming positive IgM results.

(10) Rubella testing

Testing for the presence of rubella IgM in pregnant women when there has been no evidence of rubella infection or contact with rubella cases is not recommended, as it is likely to produce false-positive results in approximately 1% of those tested as the specificity of the Siemens assay is approximately 98%–99%.

(11) Communications and sharing data

Laboratory and immunization/surveillance colleagues are encouraged to have regular interactions and meetings to share the information, to ensure classification of suspected cases and to harmonize laboratory and surveillance data.

Monthly updates of genotypes circulating in the Region should be shared with network laboratories as well as key global network laboratories (e.g. US CDC). Network laboratories are requested to provide feedback on the laboratory data included in the Measles and Rubella Bulletin.

(12) Follow-up training

A follow-up training for molecular capacity to further improve the laboratory performance should be considered during third or fourth quarter of 2012. This training will reinforce the use
of molecular techniques covered in the previous workshops and introduce a quality control programme for molecular methods and real-time RT-PCR.

(13) Modification of checklists

The accreditation checklist of the measles laboratory network will be modified to reflect the additional functions that some national or provincial laboratories have in supporting subnational or prefecture-level laboratories. The changes will include the requirement for the national or provincial laboratory to record the workload, proficiency test and confirmatory testing results of the individual subnational or prefecture laboratories and report on the timeliness of reporting confirmatory test results.
### TENTATIVE TIMETABLE

**As of 30 August**

<table>
<thead>
<tr>
<th>Time</th>
<th>Monday, 5 September</th>
<th>Time</th>
<th>Tuesday, 6 September</th>
<th>Time</th>
<th>Wednesday, 7 September</th>
<th>Time</th>
<th>Thursday, 8 September</th>
<th>Time</th>
<th>Friday, 9 September</th>
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<tbody>
<tr>
<td>0830-0900</td>
<td>1. Opening session</td>
<td>0900-1000</td>
<td>8. Experience of polio laboratory network for the lab diagnosis of hand, foot and mouth disease and other enteroviruses</td>
<td>0900-1000</td>
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<td>20. Overview of global and regional measles elimination and rubella control initiatives</td>
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<tr>
<td>0900-0930</td>
<td>Coffee break</td>
<td>0900-1000</td>
<td>10. Detection of vaccine-derived poliomyelitis virus (VDPV)</td>
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<tr>
<td>0930-1015</td>
<td>Q&amp;A</td>
<td>0900-1000</td>
<td>11. Experience with real time PCR and sequencing among IDT countries</td>
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<td>1015-1045</td>
<td>COFFEE BREAK</td>
<td>0930-1000</td>
<td>12. Investigation of Measles outbreaks and the role of the laboratory</td>
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<tr>
<td>1130-1210</td>
<td>Conclusions</td>
<td>1045-1115</td>
<td>23. Enhancing molecular surveillance</td>
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<tr>
<td>1130-1200</td>
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<td>1115-1230</td>
<td>24. Measles serosurvey</td>
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**SESSION I: Poliomyelitis Laboratory Network**

- Global Polio Eradication Initiative and maintenance of poliomyelitis-free status in the Western Pacific Region
  - Global Polio Eradication Initiative and regional updates to maintain poliomyelitis-free status
  - Status of global poliomyelitis laboratory network
  - Regional updates of poliomyelitis laboratory network

- Detection of poliovirus from non-AFP cases
  - Environmental surveillance of poliovirus and non-polio enteroviruses in China
  - Australia experience
  - Japan experience

- Experience of polio laboratory network
  - Hand, foot and mouth disease surveillance in China
  - Australia experience
  - Japan experience

- Detection of poliovirus from non-AFP cases
  - Environmental surveillance of poliovirus and non-polio enteroviruses in China
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<td>1210-1310</td>
<td><strong>LUNCH BREAK</strong></td>
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<td>1310-1340</td>
<td>5. Laboratory quality assurance</td>
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<td>(a) Report on proficiency testing: Virus isolation (20 mins)</td>
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<td>(b) Reporting on intratypic differentiation (ITD) testing</td>
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<td>(c) Laboratory quality assurance of China polio laboratory network</td>
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<td>(d) Monitoring of cell sensitivity testing</td>
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<td>(e) Accreditation of sequencing laboratories</td>
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<td>Q&amp;A</td>
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<td>6. Country reports of national polio laboratories</td>
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<td>(a) Hong Kong (China)</td>
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<td>(b) Malaysia (15 mins)</td>
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<td><strong>COFFEE BREAK</strong></td>
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<td>1540-1650</td>
<td>12. Module 2: Equipment</td>
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<td>(c) Mongolia</td>
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<td>(d) New Zealand</td>
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<td>(e) Philippines</td>
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<td>(f) Republic of Korea</td>
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<td>(g) Singapore</td>
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<td>(h) Viet Nam-northern</td>
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<td>Q&amp;A</td>
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<td>Distribution of biosafety training modules to trainers (18)</td>
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<td><strong>INFORMAL GET-TOGETHER</strong></td>
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<tr>
<td>1800</td>
<td><strong>INFORMAL GET-TOGETHER</strong></td>
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### 1300-1400

- Registration of new participants
- Session II Biosafety training

### 1400-1530

- 10. Introduction to biosafety training: (a) Introduction to biosafety training modules (b) Introduction of groups and trainer and rapporteur for each group
- Move to Rm 210 and 212

### 1500-1515

- 11. Module 1: Disinfection and waste management
- Q&A

### 1510-1540

- 12. Module 2: Equipment
- 17. Report by rapporteurs of each group (10 mins each)
- 18. Discussions: Current status and issues in WPR countries (maintaining biosafety cabinets and training, etc.)
- 19. Conclusions and recommendations of session I and II

### 1515-1545

- Distribution of polio ITD and sequencing PT samples and Biosafety training CD

### 1600-1700

- 24. Country reports:
  - (a) Cambodia
  - (b) Fiji
  - (c) Lao People’s Democratic Republic
  - (d) Macao (China)
  - (e) Malaysia
  - (f) Mongolia
  - (g) New Zealand
  - (h) Papua New Guinea
- Q&A

### 1645-1700

- Discussions on country reports
- 25. Use of alternative sampling:
  - (a) Use of dried blood spots (DBS) oral fluid and POC
- 26. Conclusions and recommendations of session III

### 1700-1730

- 27. Closing session
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8-9 September 2011
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<td>JAPAN</td>
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