REPORT

FOURTH MEETING ON VACCINE-PREVENTABLE DISEASES LABORATORY NETWORKS IN THE WESTERN PACIFIC REGION

Manila, Philippines
11–15 March 2013

Convened by:

WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

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NOTE

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

The Fourth Meeting on Vaccine-Preventable Diseases (VPD) Laboratory Networks in the Western Pacific Region was held in Manila, the Philippines from 11 to 15 March 2013 to review the performances and identify the challenges of polio, measles/rubella and Japanese encephalitis (JE) network laboratories in the Region. 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 third VPD laboratory networks meeting held in September 2011. The meeting also provided an opportunity to discuss ways to further improve the timeliness of laboratory testing among network laboratories including subnational laboratories in China.

Around 121 participants from network laboratories, advisers, observers and WHO staff attended the meeting. Participants included 80 representatives from 16 countries (12 polio laboratories, 18 measles/rubella network laboratories, and 10 JE network laboratories); two WHO laboratory coordinators from Headquarters; four advisers from the United States Centers for Disease Control and Prevention (US CDC); one adviser from Victorian Infectious Diseases Reference Laboratory (VIDRL), Australia; one adviser from National Institute of Infectious Diseases (NIID), Japan; one adviser from Health Protection Agency (HPA), United Kingdom of Great Britain and Northern Ireland; one adviser from Mahosot Hospital, Lao People's Democratic Republic; one observer from the Department of Health, Philippines; three observers from NIID, Japan; two observers from Korea Centers for Disease Control and Prevention (Korea CDC); two observers from Taipei (China); one observer from the Program for Appropriate Technology in Health (PATH), India; four observers from the Research Institute for Tropical Medicine (RITM), Philippines; five WHO country Expanded Programme on Immunization (EPI) or laboratory focal points; and 13 staff from the WHO Regional Office for the Western Pacific Region.

The objectives of the meeting were:

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

2. to identify the challenges and define/develop plans for the expanding roles of the polio network laboratories to maintain polio-free status in the Region;

3. to review the progress and identify challenges of measles/rubella laboratories, and to present and discuss the Regional Committee resolution WPR/RC63.R5 on measles elimination and acceleration of rubella control and the regional guidelines to verify measles elimination;

4. to develop plans to further strengthen the molecular detection capacity and to ensure the performance of network laboratories to support measles elimination and rubella control goals in the Region;

5. to review the progress of the recently established JE laboratory network in the Region;
(6) to develop a plan to sustain the activities of the JE laboratory network and further strengthen the performance of network laboratories to support JE control in the Region; and

(7) to discuss coordination of quality assurance-related activities of network laboratories, integration of the laboratory aspects of other communicable diseases with existing networks and how to ensure the sustainability of the networks.

Session I. Polio Laboratory Network

A two-day session for the polio laboratory network in the Western Pacific Region 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, to review the performances of the polio network laboratories and to discuss the implementation of new requirements. The session included presentations on the polio endgame strategy and updates on the polio eradication initiative, detection of vaccine-derived poliovirus (VDPVs), experiences with real-time reverse transcription polymerase chain reaction (rRT-PCR) and sequencing among ITD laboratories, laboratory quality assurance, detection of polioviruses from non-acute flaccid paralysis (AFP) cases, experiences of the polio laboratory network for the laboratory diagnosis of hand, foot and mouth disease (HFMD), biorisk management, data management and country reports.

The meeting concluded that the performance of the regional polio laboratory network has been sustained at polio-free-certification standard and that AFP surveillance activities have been efficiently supported. The network laboratories provided critical evidence in support of the continued polio-free status of the Region. As of March 2013, all 43 network laboratories are fully accredited including all seven polio laboratories with ITD function. All seven ITD laboratories passed the ITD proficiency test (PT), and five ITD laboratories scored 100%. Five ITD laboratories also participated in polio sequencing PT, and four ITD laboratories scored 100%. It is planned that seven laboratories will participate in polio sequencing PT in 2013.

Since the Western Pacific Region has been polio-free for more than 10 years, network laboratories have been actively involved in supplementary enterovirus or environmental surveillance. In particular, China established a very extensive HFMD laboratory network based on existing polio and measles/rubella laboratories, which provided support to Cambodia during the HFMD outbreak in 2012. The polio laboratories in Australia and Malaysia were involved in testing samples from environmental surveillance, and China’s polio network laboratories expanded environmental surveillance to 11 provinces. Since the definition of type 2 VDPV was changed 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 since 2010. 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.

Tremendous efforts have been made to achieve the progress towards polio eradication with support from the polio laboratory network. Great work has been done by the Global Polio Laboratory Network in terms of introducing new technologies, new methodologies and a new algorithm in support of the global polio eradication initiative. Continuous strong quality assurance procedures and training activities are being implemented in ensuring trusted high-quality laboratory support.
Session II. 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, enhancing molecular surveillance, use of alternative sampling methods, strengthening rubella virus surveillance and the role of the laboratory, measles serosurveys, data management and reporting, laboratory diagnosis of mumps and country reports.

The meeting concluded that measles and rubella network laboratories helped 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 (GSL) in Japan, three regional reference laboratories (RRLs) in Australia, China and Hong Kong (China), 13 fully functional national measles and rubella laboratories, 31 provincial and 331 prefectural laboratories in China, and three new subnational laboratories in Malaysia and Viet Nam. Among 48 laboratories for which WHO conducted on-site reviews for accreditation, 46 laboratories are fully accredited as of March 2013. Two laboratories with pending accreditation status need to improve laboratory performance by implementing recommendations from the previous accreditation visit.

As the role of the measles and rubella laboratory network also extends to molecular surveillance, laboratories with virus isolation, molecular diagnostics and sequencing capabilities were encouraged to perform virus isolation, sequencing and genotyping. Genotype and sequence information for measles are submitted to the WHO sequence database for measles, MeaNS, and rubella sequences can be submitted to the database for rubella, RubeNS, as soon as it is available. Genotype data on recent measles virus strains are available from all countries except the Pacific island countries.

In 2011-2012, the Hong Kong (China) RRL provided excellent support to identify genotypes of measles and rubella viruses circulating in Cambodia, the Lao People's Democratic Republic, Macao (China), Malaysia, Mongolia, the Philippines and Viet Nam using serum samples or virus isolates. Additional rubella genotyping was conducted for rubella-positive serum samples from Fiji, Papua New Guinea and other Pacific island countries.

Laboratories with the capacity to conduct virus isolation and molecular detection were further trained during a follow-up training in October 2012 in Hong Kong (China). Since the training, more network laboratories have become involved in virus isolation and molecular detection of measles and rubella viruses.

The network should continue to make full efforts to obtain genotype and sequence information on measles and rubella viruses circulating in the Region, as laboratory performance will be a critical component of verifying measles elimination in the countries. The recently published global framework for verification of measles and rubella elimination (Weekly Epidemiological Record, March 2013) describes five lines of evidence for determining whether a country or region has achieved measles and/or rubella elimination. Of these five lines of evidence, two are directly related to laboratory activities: the presence of high-quality epidemiologic and laboratory surveillance systems and genotyping evidence that measles and rubella virus transmission is interrupted.

The laboratories should regularly communicate and collaborate with the national surveillance or epidemiology groups and the WHO Regional Office for the Western Pacific 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 for the Western Pacific.

Session III. Japanese Encephalitis Laboratory Network

A one-day session of the regional JE laboratory network was organized to review the progress and identify challenges of the JE network laboratories and to develop plans to further strengthen the performance of network laboratories in support of JE control in the Region. The session included presentations on regional JE/acute encephalitis syndrome (AES) surveillance, JE vaccination and control initiatives, laboratory performance, quality assurance and country reports.

The Western Pacific Region has seven countries either known to be endemic for JE or suspected to be endemic for JE. These countries are Cambodia, China, the Lao People's Democratic Republic, Malaysia, Papua New Guinea, the Philippines and Viet Nam. Until recently, however, the activities of laboratories in these JE-endemic or suspected JE-endemic countries have been limited. Therefore, it was proposed in 2008 to create a JE laboratory network to improve the capability of JE case confirmation and improve the quality of JE testing among countries either known or suspected to be endemic for JE in the Western Pacific Region. The recent commercial availability of sensitive, highly specific, easy-to-perform JE antibody tests has facilitated the implementation of JE surveillance in low-resource settings.

The WHO JE laboratory network in the Western Pacific Region was based on the model of the polio and measles/rubella laboratory networks, as recommended by the Technical Advisory Group (TAG) on Immunization and Vaccine-Preventable Diseases in 2008, with support from PATH and Korea CDC. The JE laboratory network consists of one GSL in Japan, two RRLs in China and the Republic of Korea, and seven national laboratories in Cambodia, the Lao People's Democratic Republic, Malaysia, Papua New Guinea, the Philippines and northern and southern Viet Nam. Most JE network laboratories were designated in the national public health institute, where laboratory testing for measles and rubella is also performed (Cambodia, the Lao People's Democratic Republic, Papua New Guinea and the Philippines). Designation of 10 subnational JE laboratories in China (Chongqing, Guangdong, Guangxi, Guizhou, Henan, Shandong, Shanghai, Sichuan, Yunnan, and Zhejiang) is ongoing.

Three JE hands-on training workshops were conducted in 2009, 2010 and 2011 to build regional capacity for JE testing, and regional JE meetings were held in 2008, 2009 and 2011. Procedures have been standardized, and in-house assays and commercial kits are being evaluated by US CDC and NIID as they become available. Quality assurance of national JE laboratories is documented through PT, confirmatory testing by RRLs and on-site reviews for accreditation. The JE network laboratories passed WHO PT from 2009 to 2012, and concordance rates from confirmatory testing improved during that period. Eight out of 10 laboratories are accredited.

Case-based and aggregate data are received at the WHO Regional Office for the Western Pacific on a monthly basis. From 2009 to 2012, 10%–30% of AES cases were confirmed as JE. A significant proportion of cases were over 15 years old. Consequently, there is evidence to introduce JE vaccine in Cambodia, the Lao People's Democratic Republic and the Philippines. Other etiologies of AES (both viral and bacterial) need to be investigated, and countries should ultimately take responsibility for supporting and integrating JE surveillance into other VPD surveillance programmes.
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<td>ABI</td>
<td>Applied Biosystems instruments</td>
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<td>AFP</td>
<td>acute flaccid paralysis</td>
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<td>BLAST</td>
<td>Basic Local Alignment Search Tool</td>
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<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<td>CDIBP</td>
<td>Chengdu Institute of Biological Products</td>
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<tr>
<td>ChLIA</td>
<td>chemiluminescent immunoassay</td>
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<td>CMIA</td>
<td>chemiluminescent microparticle immunoassay</td>
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<td>CMV</td>
<td>cytomegalovirus</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CPHL</td>
<td>Central Public Health Laboratory</td>
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<td>CRS</td>
<td>congenital rubella syndrome</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>CV</td>
<td>coxsackievirus</td>
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<td>Ct</td>
<td>threshold cycle</td>
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<td>DBS</td>
<td>dried blot spot</td>
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<td>DPT</td>
<td>diphtheria, pertussis, and tetanus</td>
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<td>EBV</td>
<td>Epstein–Barr virus</td>
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<td>EIA</td>
<td>enzyme immunoassay</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EPI</td>
<td>Expanded Programme on Immunization</td>
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<td>ESR</td>
<td>Environmental Science and Research</td>
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<td>EV</td>
<td>enterovirus</td>
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<td>FTA</td>
<td>Fast Technology for Analysis of nucleic acids</td>
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<td>GCC</td>
<td>Global Certification Commission</td>
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<td>GPEI</td>
<td>Global Polio Eradication Initiative</td>
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<td>GPLN</td>
<td>Global Polio Laboratory Network</td>
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<td>GSL</td>
<td>global specialized laboratory</td>
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<td>HBV</td>
<td>hepatitis B virus</td>
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<td>HBsAg</td>
<td>hepatitis B surface antigen</td>
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<td>HepB3</td>
<td>three doses of Hepatitis B vaccine.</td>
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<td>HEV</td>
<td>human enterovirus</td>
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<td>HFMD</td>
<td>hand, foot and mouth disease</td>
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<td>HHV</td>
<td>human herpesvirus</td>
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<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HPA</td>
<td>Health Protection Agency</td>
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<td>IB-VPD</td>
<td>invasive bacterial vaccine-preventable disease</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
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<td>immunoglobulin M</td>
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<td>IHC</td>
<td>in-house control</td>
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<td>IMR</td>
<td>Institute of Medical Research</td>
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<td>IPV</td>
<td>inactivated polio vaccine</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>ITD</td>
<td>intratypic differentiation</td>
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<td>ISO</td>
<td>International Organization for Standardization</td>
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<td>JE</td>
<td>Japanese encephalitis</td>
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<td>L20B</td>
<td>a mouse cell line (L-cells), genetically engineered to express the human poliovirus receptor</td>
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<td>LQC</td>
<td>laboratory quality control</td>
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<td>MCV</td>
<td>measles-containing vaccine</td>
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<td>MCV1</td>
<td>first dose of measles-containing vaccine</td>
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<td>MCV2</td>
<td>second dose of measles-containing vaccine</td>
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<td>MeaNS</td>
<td>Measles Nucleotide Surveillance</td>
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<td>MEIA</td>
<td>microplate enzyme immunoassay</td>
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<td>MMR</td>
<td>measles, mumps and rubella</td>
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<td>NAPH</td>
<td>nucleic acid probe hybridisation</td>
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<td>NCC</td>
<td>National Certification Committee</td>
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<td>NCCD</td>
<td>National Center for Communicable Diseases</td>
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<td>NCLE</td>
<td>National Center for Laboratory and Epidemiology</td>
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<td>NCR</td>
<td>National Capital Region</td>
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<td>NESVPD</td>
<td>national epidemiological surveillance of vaccine-preventable diseases</td>
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<td>NIHE</td>
<td>National Institute of Hygiene and Epidemiology</td>
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<td>NIID</td>
<td>National Institute of Infectious Diseases</td>
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<td>NIPH</td>
<td>National Institute for Public Health</td>
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<td>NIR</td>
<td>National Immunisation Register</td>
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<td>NPEV</td>
<td>non-polio enterovirus</td>
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<td>NPL</td>
<td>national polio laboratory</td>
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<td>OD</td>
<td>optical density</td>
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<td>OPV</td>
<td>oral polio vaccine</td>
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<td>OPV2</td>
<td>type 2 component of oral polio vaccine</td>
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<tr>
<td>bOPV</td>
<td>bivalent oral polio vaccine</td>
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<tr>
<td>mOPV2</td>
<td>monovalent polio vaccine type 2</td>
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<tr>
<td>PATH</td>
<td>Program for Appropriate Technology in Health</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PHLC</td>
<td>Public Health Laboratory Centre</td>
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<td>PHERI</td>
<td>provincial health and environmental research institute</td>
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<td>PoCT</td>
<td>point-of-care test</td>
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<td>PRNT</td>
<td>plaque reduction neutralization test</td>
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<td>PT</td>
<td>proficiency test</td>
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<td>RCC</td>
<td>Regional Certification Commission</td>
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<td>RCV</td>
<td>rubella- containing vaccine</td>
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<td>RD</td>
<td>Rhabdomyosarcoma cells</td>
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<td>RI</td>
<td>routine immunization</td>
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<tr>
<td>RITM</td>
<td>Research Institute for Tropical Medicine</td>
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<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>RRL</td>
<td>regional reference laboratory</td>
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<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
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<td>RubeNS</td>
<td>Rubella Nucleotide Surveillance</td>
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<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>SAGE</td>
<td>Strategic Advisory Group of Experts on Immunization</td>
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<tr>
<td>SIA</td>
<td>supplemental immunization activity</td>
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<tr>
<td>hSLAM</td>
<td>human signaling lymphocytic activation molecule</td>
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<tr>
<td>SLIPTA</td>
<td>Strengthening Laboratory Quality Improvement Process Towards Accreditation</td>
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<td>SLMTA</td>
<td>Strengthening Laboratory Management Towards Accreditation</td>
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<td>TAG</td>
<td>Technical Advisory Group</td>
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<td>tOPV</td>
<td>trivalent oral polio vaccine</td>
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<td>UK</td>
<td>United Kingdom</td>
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<td>US CDC</td>
<td>United States Centers for Disease Control and Prevention</td>
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<tr>
<td>VAPP</td>
<td>vaccine-associated paralytic poliomyelitis</td>
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<td>VDPV</td>
<td>vaccine-derived poliovirus</td>
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<tr>
<td>aVDPV</td>
<td>ambiguous vaccine-derived poliovirus</td>
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<tr>
<td>cVDPV</td>
<td>circulating vaccine-derived poliovirus</td>
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<tr>
<td>iVDPV</td>
<td>immunodeficiency-related vaccine-derived poliovirus</td>
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<td>VIDRL</td>
<td>Victorian Infectious Diseases Reference Laboratory</td>
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<td>VPD</td>
<td>vaccine-preventable disease</td>
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Keywords:
/Poliomyelitis-prevention and control/Measles-prevention and control/Rubella-prevention and control/Laboratories/Vaccines/Quality control/Encephalitis, Japanese-prevention and control/
1. INTRODUCTION

1.1 Background information

The polio and measles/rubella laboratory networks have played a crucial role in maintaining polio-free status and in progressing towards achieving the regional goal of measles elimination by 2012 and accelerated rubella control by 2015 in the Western Pacific Region by providing timely and reliable laboratory confirmation and virus identification. In addition, the Japanese encephalitis (JE) laboratory network has been established to improve the capability of JE case confirmation in the Region as recommended by the Seventeenth Meeting of the Technical Advisory Group (TAG) on Immunization and Vaccine-Preventable Diseases (VPD) held in 2008.

The Expanded Programme on Immunization (EPI) organized three integrated VPD laboratory network meetings for polio, measles/rubella and JE network laboratories in July 2008, February 2010 and September 2011 to share experiences and to consider integration of activities related to quality assurance of network laboratories. High levels of laboratory performance have been maintained for VPD laboratories through a well-established quality assurance system that includes annual WHO accreditation. Molecular detection capacity has been strengthened in polio and measles/rubella network laboratories, and timely molecular data have been provided to the national surveillance and immunization programmes.

Despite the progress and success of VPD laboratory networks in the Region, their high-quality performance can only be maintained by addressing a few remaining issues and challenges, namely, expansion of the intratypic differentiation (ITD) capacity of polio laboratories, improving the collection of appropriate samples for detection of measles and rubella viruses, and sharing of measles and rubella genotyping information with the World Health Organization (WHO) and the Measles Nucleotide Surveillance (MeaNS) database.

The Fourth Meeting on Vaccine-Preventable Diseases Laboratory Networks in the Western Pacific Region was held in Manila, the Philippines from 11 to 15 March 2013 to provide global and regional updates on VPD laboratory networks in the Region, to identify challenges faced by these VPD laboratory networks, and to improve the quality of VPD laboratory networks in the Region to maintain polio-free status, to enhance the quality of measles and rubella network laboratories, and to support JE control in the Region. The meeting was organized in three parts: polio network laboratories on 11–12 March 2013, measles and rubella network laboratories on 13–14 March 2013, and JE network laboratories on 15 March 2013.

1.1.1 Global Poliomyelitis Laboratory Network (GPLN)

GPLN, which comprises global specialized laboratories (GSLs), regional reference laboratories (RRLs), and 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 GSL in Japan, two RRLs in Australia and China, nine national laboratories and 31 provincial laboratories in China. In addition to the GSL and RRLs, four national polio laboratories in the Region, namely, Hong Kong (China), Malaysia, New Zealand and Singapore, can perform ITD of polioviruses to differentiate wild, Sabin-like and vaccine-derived polioviruses.
Timely detection and identification of wild polioviruses and vaccine-derived poliovirus (VDPV) is crucial to the global polio eradication initiative, as it allows rapid response and prevents further spread of viruses. In this context, a new WHO algorithm for poliovirus isolation and identification was introduced that requires network laboratories to deliver results of primary isolation within 14 days of receipt of samples; the previous requirement was 28 days. Network laboratories are also now required to submit ITD results within seven days; this was reduced from 14 days.

In August 2011, China’s polio laboratory network detected wild poliovirus type 1 importation from Pakistan and quickly provided the results of rapid virus identification to the national programme. The molecular diagnosis method was used in parallel with the standard virus isolation method to identify the virus. After the importation of wild poliovirus type 1 that evolved into an outbreak in Xinjiang province, China’s polio laboratory network began introducing the new algorithm for virus isolation among all provinces and real-time polymerase chain reaction (PCR) for ITD of polioviruses and vaccine-derived poliovirus among selected provincial laboratories.

To further expand ITD laboratories in this Region, the Second Regional Hands-on Training Course to Implement Real-Time Polymerase Chain Reaction for Rapid Detection and Characterization of Polioviruses was organized from 3 to 7 December 2012 in Manila, the Philippines. It is expected that after this training, most polio laboratories in the Region will adopt the real-time PCR technique for ITD and VDPV screening. As a result, the time needed for ITD testing will be shortened, and the frequency of shipping virus isolates to RRLs or GSL for ITD and sequencing will be reduced. Ultimately, ITD results of polioviruses will be available earlier for appropriate programme actions.

Since the Western Pacific Region has been polio-free for more than 10 years, network laboratories in the Region have been actively involved in supplementary enterovirus surveillance. In particular, China has established a very extensive hand-foot-and-mouth disease (HFMD) laboratory network. Supplementary environmental surveillance of polio also has been strengthened in the Region to sustain high performance levels.

1.1.2 Measles and Rubella Laboratory Network

The WHO measles and rubella laboratory network in the Western Pacific Region consists of one GSL in Japan, three RRLs in Australia, China and Hong Kong (China), 16 national laboratories, 31 provincial laboratories and 331 prefecture laboratories in China, two subnational laboratories in Viet Nam and one subnational laboratory in Malaysia. With the addition of three subnational laboratories in Viet Nam and Malaysia in 2011, the total number of measles and rubella network laboratories has grown to 385 in the Region.

The WHO measles and rubella laboratory network has played a critical role in monitoring the progress of measles elimination and rubella control by confirming measles and rubella cases, as well as establishing genotyping and providing molecular epidemiological data for decision-makers in national immunization programmes. Providing timely, reliable results is critical in order to identify and respond to imported or endemic measles transmission, particularly as the Region approaches the measles elimination goal.

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 for measles is submitted to MeaNS by national or regional reference laboratories. A database for rubella genotype and sequencing information is being developed.

Following three regional hands-on training workshops on the molecular diagnosis of measles and rubella in 2009, 2010 and 2012, the Region's capacity to detect circulating genotypes has improved dramatically. Genotype information on circulating measles and rubella virus strains is available in most countries in the Region except Pacific island countries. The measles and rubella laboratory network will continue to monitor the circulating genotypes of measles and rubella viruses to provide evidence of measles elimination and rubella control in the Region. Two measles strains, genotypes H1 and D9, are still circulating in some countries in the Region.

1.1.3 Japanese Encephalitis Laboratory Network

In 2008-2009, the JE laboratory network was established to improve JE case confirmation among countries either known or suspected to be endemic for JE in the Western Pacific Region, as recommended by the Seventeenth Meeting of TAG on Immunization and Vaccine-Preventable Diseases in 2008. The network consists of one GSL in Japan, two RRLs in China and the Republic of Korea, and seven national laboratories in Cambodia, the Lao People’s Democratic Republic, Malaysia, Papua New Guinea, the Philippines and Viet Nam (northern and southern).

To build regional laboratory capacity for JE testing, three regional hands-on training workshops were organized in 2009, 2010 and 2011. For quality assurance of the JE laboratory network, annual proficiency tests were successfully conducted in 2009, 2010 and 2011, and a confirmatory testing mechanism was also established in the Region. A WHO accreditation programme for JE laboratories was initiated in 2010. Eight out of the 10 network laboratories have been accredited as of September 2012.

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 especially for ITD laboratories in the Region;

(2) to identify the challenges and define/develop plans for the expanding roles of polio network laboratories to maintain polio-free status in the Region;

(3) to review the progress and identify challenges of measles and rubella laboratories, and to present and discuss the Regional Committee resolution WPR/RC63.R5 on measles elimination and acceleration of rubella control and the regional guidelines to verify measles elimination;

(4) to develop plans to further strengthen the molecular detection capacity and to ensure the performance of network laboratories to support measles elimination and rubella control goals in the Region;

(5) to review the progress of the recently established JE laboratory network in the Region;
(6) to develop a plan to sustain the activities of the JE laboratory network and further strengthen the performance of network laboratories to support JE control in the Region; and

(7) to discuss coordination of quality assurance-related activities of network laboratories, integration of the laboratory aspects of other communicable diseases with existing networks and how to ensure the sustainability of the networks.

1.3 Appointment of workshop officers for the polio, measles and rubella, and JE laboratory networks meeting sessions

Dr Bruce Thorley was appointed as chairperson, Dr Hiroyuki Shimizu as vice-chairperson, and Dr Sue Huang as rapporteur for the polio session.

Dr Janice Lo was appointed as chairperson, Dr Xu Wenbo as vice-chairperson and Ms Vicki Vasiliki Stambos as rapporteur for the measles and rubella session.

Dr Ishiro Kurane was appointed as chairperson, Dr Lian Goudong as vice-chairperson and Dr Amado Tandoc III as rapporteur for the JE session.

1.4 Organization

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

Table 1. Participants of the Fourth 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: Measles and rubella laboratory network</th>
<th>Session III: JE laboratory network</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1</td>
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<td>-</td>
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<td>Brunei Darussalam</td>
<td>-</td>
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<td>-</td>
<td>1</td>
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<tr>
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<td>3</td>
<td>6</td>
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<tr>
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<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Hong Kong (China)</td>
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<td>2</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lao People’s Democratic Republic</td>
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</tr>
<tr>
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<tr>
<td>Philippines</td>
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<td>3</td>
<td>2</td>
<td>7</td>
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<tr>
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<td>3</td>
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<tr>
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</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Viet Nam</td>
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<td>4</td>
</tr>
</tbody>
</table>
1.5 Participants

Session I: The poliomyelitis laboratory network meeting was held for two days, from 11 to 12 March 2013, to review the performances of the laboratories, to identify challenges and ways to improve the quality of the performances, and to discuss the implementation of the recommendations from the network's meeting in September 2011. Participants included 18 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), the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Australia, and the Health Protection Agency (HPA) in the United Kingdom of Great Britain and Northern Ireland.

Session II: The measles and rubella laboratory network meeting, which took place from 13 to 14 March 2013, was attended by 40 representatives from measles laboratories in 16 countries as well as temporary advisers from US CDC, VIDRL and HPA.

Session III: The Japanese encephalitis laboratory network meeting, which took place on 15 March 2013, was attended by 22 representatives from JE laboratories in nine countries as well as temporary advisers from US CDC, HPA, the National Institute of Infectious Diseases (NIID) in Japan, and Mahosot Hospital in Vientiane, the Lao People's Democratic Republic.

Observers from HPA, NIID, the Research Institute for Tropical Medicine (RITM) and National Epidemiology Center in the Philippines, Korea Centers for Disease Control and Prevention (Korea CDC), Program for Appropriate Technology in Health (PATH) in New Delhi, and Taiwan CDC (China) also participated in the meeting.

1.6 Opening session

Dr Sergey Diorditsa, EPI Team Leader, welcomed the country participants and advisers from partner organizations. The Director of Combating Communicable Diseases, Dr John Ehrenberg, opened the meeting. He acknowledged the close collaboration of polio, measles/rubella and JE network laboratories with national EPI and surveillance programmes in each Member State, which has greatly contributed to maintaining the Region’s polio-free status and making progress towards measles elimination and rubella and JE control in the Western Pacific Region.
2. PROCEEDINGS

2.1 Polio Laboratory Network

2.1.1 Polio endgame strategy and regional updates on maintaining poliomyelitis-free status in the Western Pacific Region

(1) Polio endgame strategy and global/regional updates on the polio eradication initiative and next steps

Ms Liliane Boualam, Technical Officer, WHO Regional Office for the Western Pacific, presented global and regional updates on the polio eradication initiative and latest polio endgame strategic plan. She noted that in February 2012, WHO removed India from the list of countries with wild poliovirus – notably the biggest achievement in polio eradication in recent history. There was tremendous progress in polio eradication in 2012, as only two out of six WHO regions, the African Region and Eastern Mediterranean Region, have reported 223 cases of wild poliovirus, 97% of which were detected from three countries, namely, Afghanistan, Pakistan and Nigeria. The number of polio type 3 cases has been decreasing since 2010, and only three polio type 3 cases were reported in the last six months of 2012 from Nigeria. As of November 2012, while the number of polio-paralysed children has decreased in Afghanistan, Chad and Pakistan, a more than 50% increase in the number of paralysed children was noted in Nigeria. As of March 2013, nine wild poliovirus type 1 cases were reported in three endemic countries, namely, Pakistan (n=5), Nigeria (n=3) and Afghanistan (n=1).

In September 2012, the United Nations Secretary-General announced his full support and declared polio eradication a top priority at a high-level polio meeting hosted by the United Nations General Assembly. On 21 January 2012, the WHO Executive Board declared polio eradication a programmatic emergency for global health and requested the Director-General to rapidly finalize the polio endgame strategic plan. The plan was developed by the Global Polio Eradication Initiative in consultation with national health authorities, global health initiatives, scientific experts, donors and other stakeholders, in response to a directive of the World Health Assembly. The Polio Eradication and Endgame Strategic Plan 2013–2018 is a comprehensive, long-term strategy that addresses what is needed to deliver a polio-free world by 2018. In November 2012, the Strategic Advisory Group of Experts on Immunization (SAGE) endorsed the major objectives of the plan and its associated milestones. After incorporating input received from the WHO Executive Board on 21–29 January 2013, the plan is in the final stages of consolidation and consultation with Member States. Figure 1 shows the objectives and timeline of the Polio Eradication and Endgame Strategic Plan.
cVDPV, circulating vaccine-derived poliovirus; IPV, inactivated polio vaccine; OPV2, type 2 component of oral polio vaccine; RI, routine immunization.

The plan provides a strategy to interrupt all wild poliovirus transmission by the end of 2014. Once wild poliovirus transmission has been stopped globally, the vaccine-derived viruses will be the only source of live polioviruses in the community and could potentially lead to the re-emergence of polio. These vaccine-viruses would continue to cause vaccine-associated paralytic poliomyelitis (VAPP) and polio outbreaks due to circulating vaccine-derived polioviruses (cVDPVs). The data showed that there were 250–500 cases of VAPP per year, 40% of which were due to Sabin type 2. In the post-eradication era, therefore, use of the oral polio vaccine (OPV) in routine immunization programmes will need to be phased out to eliminate these risks. She discussed the preconditions for cessation of the type 2 component of OPV (OPV2), including prompt cVPDV2 detection and outbreak response capacity, laboratory containment and vaccine availability.

SAGE’s recommendation to introduce at least one inactivated polio vaccine (IPV) dose prior to cessation of OPV2 was discussed. Based on scientific evidence, it is expected that IPV will prevent polio if exposed to VDPV2 or wild poliovirus type 2, improve response to monovalent OPV2 (mOPV2) in an outbreak, reduce transmission of a reintroduced type 2 poliovirus, and boost immunity to wild poliovirus types 1 and 3. Rationale for the introduction of at least one dose of IPV prior to OPV2 cessation are: (1) possibility of continued silent circulation of cVDPV2 and of new cVDPV2 emergences following OPV2 cessation in countries where there are a lot of gaps in surveillance; and (2) low but real risk of type 2 polio outbreaks following OPV2 cessation (i.e. due to cVDPV, chronic VDPV excretors associated with immunodeficiency (iVDPV) and containment failure). It was noted that among the 130 countries using OPV, 18 are countries of the Western Pacific Region.

Environmental surveillance is being implemented in 15–20 additional countries to document cessation of polio type 2 circulations. Acute flaccid paralysis (AFP) surveillance should also be strengthened with particular emphasis on certified regions for the 2015 switch from OPV to IPV. Prerequisites and timelines for OPV2 removal were presented. The global certification of wild poliovirus eradication and verification of the elimination of vaccine-related viruses require highly sensitive surveillance; thus, all countries should have certification of interruption of wild poliovirus transmission, and containment of all residual wild polioviruses.
The Global Certification Commission (GCC) will consider concluding type 2 eradication based on 10 years or more of surveillance, and requires near-term reconstitution of Regional Certification Commissions (RCCs) and GCC. Major challenges for the implementation of this plan include interruption of wild poliovirus; global synchronization of OPV2 cessation; and wild poliovirus certification, VDPV verification and containment. Funding the Polio Eradication and Endgame Strategic Plan will cost the global community US$ 5.5 billion.

(2) Global wild poliovirus transmission and status of GPLN

Dr Ousmane Diop, Global Polio Laboratory Network Coordinator from WHO Headquarters, presented an update on wild poliovirus transmission and the status of GPLN. A significant decrease in polio cases worldwide from 1988 to 2012 was noted. The last case of wild poliovirus type 2 was detected in 1999. Tremendous efforts have been made to achieve this progress with support from the polio laboratory network. Significant work has been done by GPLN in recent years in terms of introducing new technologies, new methodologies, and a new algorithm to support the Global Polio Eradication Initiative. Continuous strong quality assurance procedures and training activities are being implemented to ensure trusted high-quality results and performance in the laboratory network.

Wild poliovirus cases reported from 2000 to 2012 also showed a remarkable decrease. In 2012, 223 wild polioviruses were detected in five infected countries (including three endemic countries) in two WHO regions – African Region and Eastern Mediterranean Region. In comparison, in 2009, 1609 wild poliovirus cases were reported from 23 infected countries (including four endemic countries). Cases of wild poliovirus type 3 dropped from 1122 in 2009 to 21 in 2012. Also, cases of wild poliovirus type 1 decreased from 1265 in 2010 to 202 in 2012. Only Nigeria and Pakistan have reported cases of wild poliovirus type 3 in the last 20 months. India has been removed from the list of endemic countries. Angola and the Republic of the Congo have been removed from the list of countries with re-established transmission.

Transmission of wild poliovirus types 1 and 3 from cases detected from June 2011 to December 2012 were discussed. In determining the transmission links based on genotypes of wild poliovirus type 1, it was noted that viruses in the Central African Republic, Chad and Niger were importations from Nigeria, and that re-established transmissions in both Angola and the Republic of the Congo belonged to the South Asian genotype, imported from India. In the Western Pacific Region, wild polioviruses in China were importations of the South Asian genotype from Pakistan. For wild poliovirus type 3, the South Asian genotype continues to circulate within Pakistan, while the West African genotype was imported from Nigeria to other parts of Western Africa. Genetic diversity of viruses that are presently circulating was also presented. This information is important when there is a decrease or increase in the genetic clusters and to monitor the disappearance of wild poliovirus.

GPLN has carried out numerous activities in support of the Polio Emergency Action Plan 2012–2013. In order to provide timely results, new testing algorithms are being implemented, ITD capacities are being strengthened and shipment of isolates for sequencing is being accelerated. Progress has also been made in the direct detection of poliovirus in stools to provide results even faster. A comprehensive quality assurance programme guarantees high-quality laboratory outputs, and technical assistance is provided where and when needed. Environmental surveillance data are available, where needed, to inform the programme. Laboratory management and accountability, procurement, and training of new staff have all been improved. Real-time tracking of performance is also in place. As of 31 December 2012, 141 (96.58%) global polio
network laboratories are fully accredited, four (2.74%) laboratories are provisionally accredited and one (0.68%) laboratory has failed the accreditation process.

Key GPLN’s projects for 2012-2013 were also presented, namely: (1) quality assurance for sequencing; (2) improving diagnostics (improved real-time PCR); (3) refinement of the protocol for spotting viral isolates and recovering high-quality ribonucleic acid (RNA) from FTA® (fast technology for analysis of nucleic acids) cards; (4) implementing standardized procedures; (5) biosafety and biosecurity; and (6) environmental surveillance. Main challenges facing GPLN include lack of self-funding by laboratories, staff attrition (even in GSLs), involvement of new actors and partners, and increasingly difficult laboratory coordination. Despite these challenges, GPLN continues to deliver timely, high-quality results to inform the programme, and to develop innovative activities and products to solve recurrent/emerging problems.

(3) Regional updates of poliomyelitis laboratory network and expansion of ITD laboratories

Dr Youngmee Jee, Regional Laboratory Coordinator, WHO Regional Office for the Western Pacific, presented updates on the activities of the regional polio laboratory network from 2011 to 2012. The laboratory network comprises 43 polio laboratories, including three RRLs (NIID in Japan, VIDRL in Australia, and Chinese Center for Disease Control and Prevention [China CDC]), nine national laboratories and 31 provincial laboratories in China. Seven of the network laboratories, including NIID, VIDRL, China CDC and national polio laboratories in Hong Kong (China), Malaysia, New Zealand and Singapore, are ITD laboratories. Since the Western Pacific Region has been polio-free for more than 10 years, polio laboratories have been involved in other activities to supplement AFP surveillance such as enterovirus surveillance, notably HFMD diagnosis, and environmental surveillance. Among the polio network laboratories, VIDRL in Australia, China CDC, the Institute of Medical Research (IMR) in Malaysia, and some prefectural laboratories in Japan are conducting environmental surveillance. Recently, Mongolia and Singapore have begun testing environmental samples. Environmental surveillance for poliovirus is important when a country shifts from OPV to IPV. A total of 8917 non-AFP stool samples and 171 environmental samples were tested by network laboratories (excluding China and Japan) in 2011–2012.

In 2012, there was a major expansion of ITD laboratories in Western Pacific Region and real-time PCR was introduced. Twenty-two out of 23 China provincial laboratories were trained to perform ITD after the Xianjiang polio outbreak in 2011; thus, a majority of these laboratories will perform ITD in 2013. Four additional national polio laboratories were trained for ITD in December 2012 and will also perform ITD after completion of the four implementation steps to gain proficiency. Quality assurance is well established in the Region. All but two polio laboratories (not including laboratories in China) passed the 2011 virus isolation PT using the new algorithm with a 100% score. The two remaining laboratories scored 95% and 90%. The 2012 virus isolation PT was distributed in February 2013, and the results will be collated soon. China’s laboratory network did not receive the 2011 PT and 2012 PT, but the 2012 PT will be provided soon and the new algorithm will be used. Seven ITD laboratories passed ITD PT in 2011–2012; five laboratories used real-time PCR and two laboratories used conventional PCR. All seven laboratories obtained a 100% score for ITD in 2011, while in 2012, five laboratories obtained a 100% score and two laboratories passed with 97.5% and 92.5% scores. Five ITD laboratories, with the exception of Malaysia and New Zealand, participated in a polio sequencing PT in 2012. All seven ITD laboratories will perform polio sequencing in 2013, and sequencing
laboratory accreditation will be conducted. Improved sharing of cell sensitivity testing results was noted.

In 2011, China detected six type 2 VDPVs from AFP cases and three type 2 VDPVs from non-AFP cases, and in 2012, six VDPVs were detected (one type 1 from Myanmar, four type 2 and one with mixed type 2 and type 3). Viet Nam has also detected two type 2 VDPVs from AFP cases. There was a marked improvement in timeliness of reporting of results using the new algorithm, jumping from 86% in 2009 to 96% in 2012. China’s polio laboratories were still using the old algorithm with 99% timeliness in reporting in 2012; however, these laboratories will shift to the new algorithm in 2013. Timeliness of reporting ITD results within seven days was 93% in 2012. Laboratories conducted biosafety training using WHO modules. Recommendations from the 2011 VPD laboratory network meeting were reviewed. A new polio laboratory database for AFP and non-AFP will be distributed, and laboratories were requested to share monthly non-AFP aggregate data (environmental and enterovirus surveillance) using the new laboratory database.

2.1.2 Detection of VDPV, outcomes of VDPV meeting and expansion of ITD laboratories

(1) Global update on VDPV detection and outcomes of the 2012 VDPV meeting

Dr Ousmane Diop presented the outcomes of the 2012 VDPV meeting and updates on global VDPV detection. He explained that the clinical picture of VDPV is indistinguishable from wild poliovirus. Both types of virus pose the same threat and require the same rapid and systematic response. VDPV must be controlled and managed by strengthening surveillance and conducting preventive supplemental immunization activities (SIAs) to raise type-specific population immunity, where appropriate and indicated. He noted that type 2 VDPV outbreaks are increasing in number and need judicious use of trivalent OPV (tOPV) in SIAs until countries switch from tOPV to bivalent OPV (bOPV) in routine immunization. In November 2012, SAGE recommended the introduction of at least one dose of IPV before OPV2 cessation because IPV has a role in immunological priming for subsequent systemic and intestinal immune responses to OPV. AFP surveillance is the most important tool to detect poliovirus, but environmental surveillance and enterovirus surveillance are important supplements, especially during post-OPV cessation and post-eradication. However, he encouraged the expansion of enterovirus sites. He also emphasized the need to improve technologies to increase the efficiency of detection of VDPV from environmental samples. To improve sensitivity, specificity and efficiency of VDPV detection and characterization, new assays are needed to directly target VDPV, and expansion of the network of environmental surveillance sites is encouraged, where appropriate.

In 2011, approximately 433 million children (adults in some places) received some kind of OPV during SIAs in 54 countries, and approximately 429 million children (adults in some places) received some kind of OPV during SIAs in 45 countries in 2012. The cVDPV in Niger (2006, 2009, 2010) and Chad (2010) was linked to the Nigeria outbreak. The cVDPV in Kenya in 2012 was linked to the Somalia outbreak. During 2008–2012, ambiguous VDPVs (aVDPVs) were also detected in Angola, Cameroon, Chad and Somalia in 2008; Afghanistan, China and India in 2009; China, Congo, India, Myanmar, Somalia, Syrian Arab Republic and Tajikistan in 2010; Argentina, Burundi, Congo, India, Nigeria, Peru and Yemen in 2011; Afghanistan, Egypt, Ethiopia, Myanmar, Nigeria, Sudan, Viet Nam and Yemen in 2012. Thirty-eight cases of cVDPV type 2 were detected in Afghanistan, Chad, Nigeria, Pakistan and Somalia from 6 September 2012 to 5 March 2013. Current hotspots for VDPV threat are Afghanistan, Chad, the Republic of the Congo, Kenya, Nigeria, Pakistan and Somalia. Laboratories within GPLN are leading efforts to improve detection of VDPVs through various surveillance systems and research projects such
as surveys on healthy or ill children. Laboratories outside GPLN are also contributing to these undertakings.

Classification and categorization of some VDPVs, and reporting and notification of all VDPVs remain as challenges. Progress continues to be made on the development of VDPV assays to further enhance detection, reporting and rapid response to VDPV emergences.

(2) VDPV surveillance during 2011–2013 in China

Dr Wenbo Xu from China CDC presented on VDPV surveillance in China during the period 2011–2012. AFP incidence has been hovering around 1.9 per 100 000 population over the last 10 years; however, in 2011, the incidence increased to over 2.5 per 100 000 due to polio importation in Xinjiang in 2011, resulting in enhanced AFP surveillance in China.

From 2000 to 2012, 29 cases of VDPV type 1, 79 cases of VDPV type 2 and 41 cases of VDPV type 3 were detected in China among AFP cases, contacts of AFP cases and healthy children. In 2010, the criteria for designation of VDPV type 2 were redefined to include all isolates with six or more nucleotide differences from the Sabin strains. Criteria for types 1 and 3 remain the same, with isolates having more than 10 nucleotide differences from the corresponding Sabin strains. With the new definition of VDPV type 2, five more cases of VDPV type 2 have been detected including a type 1 aVDPV case in Yunnan province imported from Myanmar in 2012. During 2011–2012, China noted an increase in the number of detected VDPV cases and the emergence of newly identified iVDPV, cVDPV and aVDPV cases in Ningxia, Sichuan and Tianjin provinces. Three iVDPV cases have been detected in China. In Anhui in 2005, a case of x-linked agammaglobulinemia caused by VDPV types 2 and 3 was detected. In 2011, iVDPV type 3 cases were detected from Ningxia from a case of common variable immunodeficiency, and in 2012, from Tianjin province, cases of iVDPV types 2 and 3 with unknown immunodeficiency diagnosis were detected.

China CDC has initiated sequencing of all Sabin-like polioviruses isolated since 2007, and a classification of pre-VDPV is being done in China for programmatic actions. Polioviruses are considered pre-VDPVs when the following criteria are met: (1) polio type 1 and type 3: from six to nine nucleotide differences of VP1 region compared with Sabin; (2) polio type 2: from three to five nucleotide differences of VP1 region compared with Sabin; and (3) at least two AFP cases caused by similar virus. Based on these criteria, several pre-VDPVs have been detected in China.

It was highlighted that high-quality surveillance, early detection by fast PCR and sequencing, early response and the current polio immunization strategy in China have been effective in preventing and controlling sustained transmission of VDPVs.

2.1.3 Reports from countries and experience with introduction of real-time PCR and sequencing of AFP and non-AFP samples

(1) Japan

Dr Hiroyuki Shimizu from NIID presented the polio laboratory activities of the institute between 2011 and 2013. He started by presenting the epidemiology of poliovirus infection since the 1950s in Japan. 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 vaccine-associated paralytic polio (VAPP) cases have been detected. Milestones associated with the introduction of IPV in Japan since 1998
were discussed. A DPT-IPV development plan was initiated in 2002 and approved in July 2012; however, standalone IPV was developed and approved in September 2012. Routine immunization using IPV started in November 2012. An apparent decline in OPV coverage in 2011–2012 was due to public health concern about the VAPP risk and vaccination of IPV products; however, there was limited access to the imported IPV products until September 2012. Age-specific OPV immunization history was analysed for each age group. To maintain polio-free status in Japan, VAPP surveillance and infectious agent surveillance, healthy children stool surveys and serosurveys to detect polio neutralizing antibody titres are being conducted. A preparedness plan for detection of and response to wild poliovirus importation and cVDPV in Japan was finalized in November 2012.

The NIID polio laboratory serves as national polio laboratory (NPL) for Cambodia and the Lao People’s Democratic Republic and as 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 183 AFP cases in Cambodia and 90 AFP cases in the Lao People’s Democratic Republic were received between January 2011 and 6 March 2013. Among those samples received from Cambodia and the Lao People’s Democratic Republic, one and 12 polioviruses were isolated, respectively. The non-polio enterovirus (NPEV) isolation rate from these samples was about 20%.

Cell sensitivity has been performed, and the results have been maintained within the acceptable range. Low passage of RD and L20B cell lines are in use. The new algorithm has been fully implemented since January 2010. Real-time RT-PCR for ITD has been employed since 2010. For sequencing, an in-house sequencing procedure, primers among others, is being used. It was noted that the US CDC sequencing procedure is being standardized and will be introduced soon at NIID.

A training course on laboratory diagnosis techniques for the control of VPDs including poliomyelitis and measles is conducted annually at NIID, and participants from China, Malaysia, the Philippines and Viet Nam were invited to the January 2011 training. It was noted that a JICA-sponsored training course will be conducted from mid-January to mid-February 2014, and that laboratory staff from the regional polio laboratory network can participate in the training.

(2) Australia

Dr Bruce Thorley from VIDRL presented and discussed immunization coverage and AFP surveillance in Australia as well as implementation of ITD real-time PCR and sequencing. He said that OPV is no longer available in Australia and that exclusive use of IPV started from November 2005 for children aged two months, four months, six months and four years old. National immunization coverage as of 30 September 2012 is high, with more than 90.0% coverage for children up to five years of age. Nevertheless, there are also areas within Australia with lower vaccine coverage.

AFP surveillance in Australia is coordinated by VIDRL and the Australian Paediatric Surveillance Unit. VIDRL also functions as the secretariat for the polio expert panel for classification of AFP cases. For the last five years, the WHO AFP surveillance performance indicator of one non-polio AFP case per 100 000 population under 15 years has been reached. Compared to many other countries, Australia has a relatively low expected number of cases, and only 43 non-AFP cases are expected in 2013. An adequate stool collection rate (i.e. 80% of AFP cases with two specimens collected more than 24 hours apart and within 14 days of onset of
paralysis) has not been achieved. The stool collection rate has been averaging around 30% since 2008, which is a concern. In 2013, the National Certification Committee formed a Stool Collection Working Group, of which VIDRL is a member, to examine and analyse the data more thoroughly and find ways to improve the stool collection rate. From 1996 to 2012, enteroviruses were isolated/detected from 40 cases, such as Sabin-like poliovirus 1, 2, 3; echovirus 3, 9, 11, 18, 19, 25, 30; coxsackievirus A4, A7, A16, A24, B2, B3, B5; and enterovirus 68, 71, 75.

Monitoring of cell sensitivity of RD and L20B cells is being done. Corrective measures are being implemented when a downward trend in sensitivity is observed. New RD and L20B cell lines, and new Sabin poliovirus reference strains from the National Institute for Biological Standards and Control were requested. New working stocks of laboratory quality-control (LQC) standards of Authenticated Sabin Poliovirus Reference Strains will be prepared, and low passages of RD and L20 B cell lines will be available. PT results on polio isolation, ITD (and VDPV) RT-PCR showed 100% scores from 2001 to 2012 (except for polio isolation in 2010, which was 95%) and a sequencing score of 100% in 2012. The laboratory is also participating in the quality assurance programme conducted by the Royal College of Pathologists of Australasia on enterovirus RT-PCR detection twice yearly.

VIDRL is also conducting laboratory investigations of AFP stool samples from Brunei Darussalam, Pacific island countries (Fiji, Nauru, Solomon Islands) and Papua New Guinea from 2002 to 2012. From 2010 to 2013, real-time RT-PCR was used for ITD testing of 47 samples from the Western Pacific Region: Brunei Darussalam (n=6), Malaysia (n=8), Papua New Guinea (n=2), and the Philippines (n=31). Thirty-eight samples were polio Sabin-like and nine samples had discordant results from ITD testing. No VDPV was detected after sequencing the nine discordant isolates. ITD results were reported within seven days.

Dr Thorley compared real-time RT-PCR and dual-stage real-time RT-PCR profiles. It is easy to introduce dual-stage real-time RT-PCR when the laboratory is already performing real-time RT-PCR because it requires changing only the cycling profile, not the reagents. It was pointed out that the cycle threshold (Ct) value with real-time RT-PCR was around 15–20, but with dual-stage real-time RT-PCR, the Ct value for Pan EV was cut to less than 10. This means that there is an increase in sensitivity because of the dual-stage nature of the amplification. With dual-stage real-time RT-PCR, the Ct value is also lower than the standard real-time PCR assay. Furthermore, nucleic acid is no longer extracted, cell lysate is diluted into half, and 1.0 µl is added into the mixture rather than 0.5 µl. It is also important in the analysis to know how to set the manual baseline and Ct value and how to use multicomponent plots when checking the raw data. If there is a need to provide Ct values in validating the diagnostic assay, avoid using Ct values less than five because it may not be computed properly by the Excel spreadsheet when importing/exporting data and may give spurious results. The same version of software (ABI 7500 v2.0.6) should be used on all computers. Yearly service maintenance of the ABI machine might call for calibration, lamp replacement and back-up. Irregular power supply was noted. It was advised to disconnect the computer from the network since anti-virus software and Windows updates could interfere with the machine for analysis.

(3) China: Report on the introduction of the new virus isolation algorithm and ITD expansion among provincial laboratories

Dr Yong Zhang from China CDC reported on the introduction of the new algorithm for virus isolation and ITD expansion among 23 provincial polio laboratories. The new algorithm for virus isolation was introduced in an annual polio workshop conducted in February 2012. In
March 2012, US CDC and China CDC conducted two training courses on real-time RT-PCR. Twenty-five laboratory staff from 23 provincial polio laboratories participated in the training. Eight provincial laboratories still need to train their staff. The 23 trained provincial laboratories in China implemented steps 1 and 2 of real-time RT-PCR preliminary experiments by the end of August 2012 and proposed to implement real-time RT-PCR by January 2013. Step 1 is conducting an experiment for three repeated runs of six samples including polioviruses Sabin1, Sabin2, Sabin3, Sabin1+2, Sabin1+3 and Sabin1+2+3. Step 2 is retrospective testing of 14 polioviruses previously isolated from individual provincial laboratories. The 23 provincial laboratories forwarded the results of the two steps to US CDC experts who provided feedback. In September 2012, China’s NPL and the 23 trained provincial polio laboratories participated in WHO PT for ITD and VDPV screening. All of the laboratories, except Guizhou provincial polio laboratory, passed the PT with a 100% score.

A notice was issued to conduct poliovirus detection using the new algorithm in China’s polio laboratory network; thus, 22 trained polio laboratories will conduct virus isolation and ITD, while eight untrained provincial polio laboratories and Guizhou polio laboratory will conduct virus isolation and refer poliovirus isolates to NPL for ITD. All polio laboratories in China’s laboratory network perform virus isolation under the new algorithm and report virus isolation results within 18 days. Polio laboratories trained to do real-time RT-PCR ITD, report ITD results to NPL within seven days after virus isolation. All L20B isolates (UN2814, Class A) are sent to NPL within 14 days after ITD; if isolates are poliovirus mixtures, neutralization tests are required while waiting for the transportation permit during this 14-day period. NPL reports the sequencing results within seven days after receipt of the L20B isolate. Un-trained polio laboratories send all L20B isolates to NPL within 14 days after virus isolation; NPL reports the ITD results and sequencing results within seven days after receipt of the L20B isolate.

From January to February 2013, there were 800 stool specimens processed for viral isolation. Timeliness in reporting is 90.5% within 14 days and 98% within 18 days. Timeliness in reporting of ITD and VDPV screening and sequencing results is 100% within seven days. The percentage of type 1 and type 3 vaccine-related polioviruses falsely flagged for further sequencing is 0% (zero out of seven and zero out of 12, respectively). The percentage of type 2 vaccine-related polioviruses falsely flagged for further sequencing is relatively high – 33.3% (four out of 12). The Sabin 2 VDPV assay is very sensitive; one nucleotide change at the probe site (VP1 amino acids 142-147) will result in a negative VP1 signal. Normal Sabin 2 viruses can have a single change at probe site and will be falsely flagged for further investigation. Timeliness in reporting will be improved when all the laboratories in China’s laboratory network are very familiar with the new algorithm. The WHO checklist will include the quality control indicator using the new algorithm for the trained provincial polio laboratories in China. The eight un-trained provincial polio laboratories wanted to upgrade their capacity to include ITD function; however, it was clarified that since only a few specimens are received per year (zero–two), it is difficult for the staff to maintain proficiency.

2.1.4 Reports from countries and areas on the national polio laboratories

(1) Hong Kong (China)

Dr Janice Lo from the Public Health Laboratory Centre (PHLC) presented the activities of the NPL in Hong Kong (China). PHLC is a major diagnostic and reference virology laboratory for Hong Kong (China) and was designated as NPL in 1992. ITD tests have been performed since 2002, and real-time RT-PCR for ITD and VDPV detection was initiated in late
2012. The laboratory has been accredited by WHO since the system began and is also accredited in accordance with ISO 15189. The NPL is the centralized virology laboratory for the diagnosis of enterovirus infections and is responsible for AFP surveillance, enterovirus surveillance and serological surveys. The AFP surveillance has met its expected number of AFP cases (>1 non-polio case per 100 000 population under 15 years of age) each year. The laboratory also receives AFP samples from Macau (China). Sixteen AFP cases were investigated in 2011 (including one from Macau [China]), and 14 were investigated in 2012. The laboratory investigated 35 stool samples in 2011 and 46 stool samples in 2012; no poliovirus was isolated, and only a few NPEVs were identified from AFP surveillance. For enterovirus surveillance, more than 60 000 clinical samples were processed, of which only around 1000 were stool samples. Over the years, few polioviruses (Sabin strains, VDPV in 2005) and NPEVs were isolated through enterovirus surveillance; however, no poliovirus was isolated in 2012 as of August. The NPEV types were coxsackievirus A, coxsackievirus B, echoviruses and EV71. Serological survey is part of the monitoring of the vaccination programme in Hong Kong (China), so every five years, neutralization tests are performed on the serum samples received in the laboratory for routine diagnostics and are saved for the purpose. The results of the last serological survey in 2010 showed high immunity (>99%) for polio type 1 and polio type 2 among all age groups and for polio type 3 among the following age groups: 1–10 years (95%), 11–20 years (93%), 21–30 years (83%), and >30 years (89%).

The results of cell sensitivity tests on L20B cells were presented. The results showed Sabin 1 and Sabin 2 were within acceptable range, while an outlier with Sabin 3 was noted. However, when the test was repeated, the result was within the acceptable limit. From 2010 to 2012, PT results for virus isolation and ITD using conventional PCR were 100%, and in 2012, the PT result for sequencing (done for the first time) was 100%.

Recently, the real-time RT-PCR technique was introduced to replace the conventional PCR for ITD testing. Among the three implementation steps recommended for ITD and VDPV detection, Steps 1 and 2 have been completed. Step 1, four test runs of a panel of 10 Sabin strains in various combinations (Sabin1; Sabin2; Sabin3; Sabin1 + Sabin2; Sabin1 + Sabin3; Sabin2 + Sabin3; Sabin1 + Sabin2 + Sabin3; NPEV [EV71]; Negative [Influenza A]) was completed in January 2013. Step 2, retrospective testing of 13 known Sabin-like polio isolates, was completed on 28 February 2013. Five samples were taken in 2008 (1 PV1-SL; 4 PV2-SL), three in 2009 (2 PV1-SL; 1 PV2-SL), one in 2010 (PV2-SL), and four in 2011 (all PV2-SL). From the five samples in 2008, three of the PV2-SL specimens were found to be a mixture containing PV1-SL as well, and from four samples in 2011, one was classified as PV2-NSL by VDPV real-time RT-PCR (VP1 sequencing revealed single mutation in probe region). As Hong Kong (China) moves forward, PHLC proposes testing specimens from the 2012 culture proficiency panel, implementing Step 3 (testing prospective L20B culture-positive isolates) and participating in real-time RT-PCR proficiency testing.

(2) Malaysia

Mr Mohd Apandi bin Yusof from IMR presented the AFP surveillance activities in his country. In Malaysia, where size of the population under 15 years old is about 9.4 million, the expected number of AFP cases per year is 94; however, there were 143 and 158 AFP cases reported in 2011 and 2012, respectively. As of February 2013, 19 AFP cases were reported. The non-AFP rate is 1.01% and more than 90% of cases had adequate stool samples since 2012. The virology unit of 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.
In Malaysia, IPV was introduced in eight states in 2008. Polio vaccine coverage is more than 90%. No case due to wild poliovirus has been detected since 1993. The NPEV rate of AFP surveillance samples remains very low at 4.9% in 2011 and 3.8% in 2012. The Ministry of Health drafted a contingency plan for the detection of wild poliovirus infection, cVDPV and clusters of AFP cases in September 2010.

The laboratory is heavily involved in the investigation of HFMD, meningoencephalitis and other infections due to enteroviruses. Samples from non-AFP cases were tested, including: 1406 samples in 2011, 1793 samples in 2012, and 235 samples in 2013 (until February). A total of two polioviruses (Sabin strains) were isolated from non-AFP cases in 2011, but no poliovirus has been isolated from all sources from 2012 to February 2013.

Cell sensitivity testing was performed on the new batch of RD and L20B cell lines from VIDRL, Australia, and the results are within acceptable range. PT results on virus isolation and identification, real-time RT-PCR ITD and VDPV screening have been consistently 100% since 2010; however, in 2012, the PT results of real-time RT-PCR ITD was 92.5% and VDPV screening was 94.5%. Biosafety training using WHO training modules was conducted from 11 October 2011 to 14 February 2012. Fifteen participants, the majority of which came from tissue culture laboratories, participated in the training. A few challenges were presented, namely, low NPEV isolation rate, need for more formal training on real-time RT-PCR to improve proficiency, and space constraints, as the new laboratory will be ready only in 2016.

(3) Mongolia

Dr Purev Suvd from the National Center for Public Health presented the surveillance activities and immunization programme in Mongolia. The size of the population under 15 years old is 797,642; thus, the expected AFP cases per year is about eight in Mongolia. The Ministry of Health approved the National Preparedness Plan for Wild Poliovirus Importation, 2010–2014. Routine OPV immunization coverage was 97.3% in 2012.

The national EPI team collects AFP surveillance reports from all provinces and the capital city weekly. Completeness of weekly AFP reporting from provinces was 97% in 2012. The NPL received a total of 20 stool samples from AFP cases in 2011 (n=11) and 2012 (n=9); no sample has been submitted yet in 2013. No poliovirus was isolated from AFP surveillance during this period. Adequacy of stool samples and timeliness in reporting have both been 100% since 2011. The NPEV rate was 18.1% in 2011 and 11.1% in 2012. The laboratory is fully accredited by WHO.

A healthy children survey is being conducted in Mongolia to supplement the AFP surveillance, and a total of 325 samples were received from 2011 to February 2013. The laboratory also received samples from other sources: 41 samples for differential diagnosis in 2011–2012 and 20 samples from environmental sources in 2012. One poliovirus (Sabin strain) was isolated from the healthy children survey. The overall NPEV rate from non-AFP samples is 22.1%. 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.

Cell sensitivity testing done on 23 August 2011 showed a decline in LQC titre; however, results of subsequent testing in 2012 were within the acceptable range. Biosafety training was organized in 2012 using the WHO-recommended training modules and was attended by 23
workers/researchers, laboratory staff and technicians. Regular training on strengthening AFP surveillance for primary health care workers and for new staff in rural areas is planned.

(4) New Zealand

Dr Sue Huang presented the NPL surveillance activities in New Zealand. In New Zealand, the size of the population of children under 15 years of age is 891 000; hence, the expected number of AFP cases is about eight or nine per year. 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 national response plan for wild polio importation was developed in 2009 and published on: http://www.health.govt.nz/publication/national-polioresponse-plan-new-zealand. OPV was used between 1960 and 2001, and since 2002, IPV has replaced OPV. The workload of the laboratory is very low due to the small number of expected AFP cases. From 2011 to 2012, 20 stool samples from 10 AFP cases were processed, and one (5%) NPEV (EV71) was isolated in 2011; no sample was received in 2013 as of February. From 2011 to February 2013, the laboratory received 6206 clinical samples, mostly respiratory samples, and only 85 were non-AFP stools. The NPEV rate of non-AFP stools was 21.2% (n=18) during this period. Cell sensitivity testing is performed regularly on RD and L20B cells, and the results are within the acceptable range; however, poliovirus type 3 in L20B cells showed a decreasing trend. Repeat testing will be done to check the media being used and results will be shared with the WHO regional laboratory coordinator.

This laboratory is also involved in national enterovirus surveillance, which supplements AFP surveillance in New Zealand. Among the 447 enteroviruses reported in 2011 were coxsackieviruses A and B, echoviruses, and EV71, EV74 and EV109. Since 1990, the PT score for viral isolation and polio ITD has been consistently 100%: results of the 2012 isolation PT were just submitted to VIDRL. Biosafety training was conducted using the WHO biosafety training modules for all polio staff and expanded to all microbiology staff in ESR–Wallaceville as well. Due to the very low workload for AFP samples, laboratory staff need to spend more time on maintaining technical capability for the laboratory diagnosis of polio. The laboratory is also planning to set up polio sequencing techniques; however, since the introduction of IPV in New Zealand, no Sabin poliovirus has been isolated from AFP surveillance that can be used in performing this method. The National Certification Committee for the Eradication of Poliomyelitis helped improve the AFP reporting rate in 2012. The Ministry of Health is delaying the implementation of environmental surveillance until AFP surveillance is improved.

(5) Philippines

Dr Amado Tandoc III from RITM presented the work done by the NPL to support AFP surveillance in the Philippines. The NPL works in close coordination with the Department of Health–National Epidemiology Center and EPI. Experts from the institute actively participate in AFP expert panel meetings for case classification, participate in Department of Health AFP/VPD advocacy meetings in the regions and provide regular feedback on performance. In 2012, the NPL started issuing a Quarterly Polio and Measles Bulletin to the Department of Health and regions. The NPL has also participated in the drafting of the country’s plan for preparedness and response to wild poliovirus importation that covers enhancement of surveillance, laboratory and immunization activities in the event of a laboratory-confirmed wild polio virus. To improve training materials for the surveillance units, the laboratory provided visual aids and video training materials on specimen collection, storage and transport. Through the Department of Health and WHO, the NPL has provided dedicated transport boxes to all the
regional surveillance units to encourage standardized shipment procedures and to discourage the use of vaccine transport boxes for stool samples. The laboratory maintains high-quality laboratory services by performing quality assurance, ensuring continuous training of staff and improving biosafety practices. The NPL was fully accredited for 2011 and 2012.

In 2012, a total of 976 stool samples were received from AFP surveillance and tested. The NPEV rate was 8%. Field performance indicators showed that in 2012, out of 17 regions, only seven regions had reached the surveillance indicator of adequate stool sample collection (80% of samples collected within 14 days from onset), compared to 2011 when all the regions had achieved over 90%. The percentage of samples transported in reverse cold chain was more than 90% in a majority of the regions. Timeliness in reporting of results was 95.4% in 2011 and again in 2012. Cell sensitivity testing on RD and L20B cells are being performed regularly and the results were within acceptable range.

All NPL and technical staff including utility and service crew and biomedical/engineer staff assigned to the virology laboratory were vaccinated using IPV. In March 2012, a two-day training course was conducted for all virology department staff using the WHO biosafety training modules. A division-level, training-of-trainers course was also conducted in February 2013 to constitute a group of more experienced trainers, who in return, will conduct biosafety training for staff of all laboratory departments of RITM.

A Second Regional Hands-on Training to Implement Real-time Polymerase Chain Reaction for Rapid Detection and Characterization of Polioviruses was conducted by US CDC and WHO at RITM from 3 to 7 December 2012. The laboratory is in the process of implementing the four steps, including final proficiency testing, and expects to be fully using the technique by June 2013.

Challenges include specimen condition (breakage and spillage in transit), storage and shipment from site to NPL (reverse cold chain), incomplete information in the case investigation forms, inadequate stool collection, insufficient feedback mechanisms (need more bulletins, e-mail transmittals, etc.), and slow transmittal to referring hospital. An area of concern is the low NPEV isolation rate (6.9% in 2011 and 8% in 2012) from AFP surveillance. The laboratory, therefore, proposes to conduct healthy children stool surveys. The laboratory is also conducting HFMD surveillance. Enteroviruses detected from HFMD surveillance are EV71, coxsackievirus A6, coxsackievirus A16, echovirus 1, echovirus 11. EV71 belongs to genotype C2. Containment of wild poliovirus and poliovirus infectious material is a challenge and will be addressed.

(6) Republic of Korea

Dr Doo Sung Cheon from the Korea CDC presented the immunization system and surveillance activities in the Republic of Korea. The estimated total population of children younger than 15 years of age is 7.56 million; thus, the AFP rate should be around 76 per year in the 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 coverage is over 95%. The non-polio AFP rate was 1.25 in 2012, and the average annual non-polio AFP rate was 0.44 from 2004 to 2011. There are 50 paediatric neurology hospitals involved in AFP surveillance, and the Catholic University is conducting an on-going AFP enhancement research project to improve the surveillance.

The NPL supports the AFP surveillance system in the Republic of Korea. In 2011, only 31 AFP cases were reported and 29 cases had adequate stool samples; in 2012, there were 96 AFP
cases reported and 93 cases had adequate stool samples. Only NPEVs were isolated, namely, EV71, CVA4, CVA6, CVB3, E6, E7 and E30. No poliovirus was isolated.

Cell sensitivity data showed satisfactory results for both RD and L20B cell lines. Results of the PT for virus isolation were consistently 100% from 2010 to 2012. The staff attended the Second Regional Hands-on Training to Implement Real-time Polymerase Chain Reaction for Rapid Detection and Characterization of Polioviruses conducted by US CDC and WHO at RITM from 3 to 7 December 2012. Step 1 of the four steps for real-time RT-PCR implementation has been implemented, and the laboratory is in the process of implementing the other steps.

In 2010, a national preparedness plan for wild polio importation was developed. Monthly training on biosafety in the laboratory is offered to NPL staff. A biosafety officer has been assigned, and biosafety manuals are available. The laboratory undergoes regular investigation by an institutional biosafety team. The only challenge for the present is setting up real-time PCR ITD.

(7) Singapore

Dr Lui Sook Yin from Singapore General Hospital presented the immunization and surveillance programmes and national polio laboratory activities in Singapore. The main role of the Virology Laboratory in the Singapore General Hospital is to provide laboratory diagnostic services for virus infections for patients in the hospital, as well as other hospitals and clinics in Singapore. It serves as WHO national laboratories for poliovirus, measles and rubella viruses.

The national childhood and adolescent polio immunization schedule covers children aged three months to 11 years old. The vaccination coverage rate is more than 90%.

The Ministry of Health established an AFP surveillance system in 1995, and the NPL in Singapore General Hospital has been WHO-accredited since 1998. The AFP surveillance system requires hospitals to notify the Ministry of Health of all patients with diseases that could lead to AFP, whether or not AFP is present. This laboratory receives samples from an AFP surveillance system, routine diagnostics as well as environmental samples. There were only four AFP cases reported in 2011, and 10 AFP cases reported in 2012. In 2011, eight AFP stool samples were received, and in 2012, 18 AFP stool samples were received in the laboratory. No AFP stool sample has been received in 2013 as of January. In addition, in 2011, 2012 and 2013 (as of January), 400 samples, 333 samples and 34 samples, respectively, were received for routine enterovirus diagnostic testing. For environmental surveillance, random samples of raw or treated reservoir or river water are collected once a week. A total of 99 environmental samples were received from 2011 to January 2013 for testing. There were 16 NPEVs isolated/detected in 2011 and 2012, consisting of CVA6, CVB1, CVB4, CVB5, E3, E6, E9, E25, E30, EV71 and EV90. No poliovirus was detected from environmental samples from 2011 to January 2013. Cell sensitivity testing is performed regularly, and the results have been very satisfactory. PT results on virus isolation were 100% in 2010 and 2011; PT results for 2012 are still pending. The real-time RT-PCR ITD PT results in 2010, 2011 and 2012 were consistently 100%, and the sequencing PT result in 2012 was 100%.

Biosafety training based on WHO biosafety training modules was conducted from 3 February 2012 to 16 March 2012, and feedback from trainees was positive. The main challenge was fulfilling the two criteria for accreditation of polio sequencing laboratory. The first criterion is the sequencing of at least 25 poliovirus and/or NPEV isolates annually, which requires a
standardized protocol for sequencing VP1 of NPEV. To meet the numbers, since there are very few prospective poliovirus and NPEV isolates each year, using retrospective archived isolates should be considered. The second criterion is reporting PT results within seven days of panel receipt. In 2012, poliovirus sequencing PT results were reported within 14 days of panel receipt.

(8) Viet Nam (Ha Noi)

Dr Nguyen Thi Hien Thanh from the National Institute of Hygiene and Epidemiology (NIHE) presented the immunization and surveillance activities in northern Viet Nam. In Viet Nam, reported routine polio immunization coverage is 96% and campaign coverage is around 97%. The rate of non-polio AFP cases in 2011 and 2012 was around 1.7% and 2.3% respectively. The laboratory received 386 AFP stool samples in 2011, 429 stool samples in 2012, and 28 stool samples in 2013 as of February. The NPEV rate was 5.4% in 2011, 11.4% in 2012, and 7.1% in 2013. NPEVs include coxsackievirus A, coxsackievirus B and echoviruses. Three Sabin-like polioviruses were isolated, one in 2011 (n=1) and two in 2012 (n=2). In 2012, two VDPV2 cases were detected in southern Viet Nam with six nucleotide differences (0.66%) from Sabin strain (confirmed by the RRL at NIID, Japan). An action plan for quick response to the detection of VDPVs in two districts was prepared: vaccine and logistics supply, human resources, budget allocation and others. Children under five years old were given two rounds of OPV. Vaccine coverage in Vinh Chau District, Soc Trang province was about 92%, while coverage in Tan Phu District, Dong Nai province was 96%. An OPV mass campaign of children under five years old in neighbouring areas or in areas where OPV3 coverage is less than 90% was carried out from October to November 2012.

Laboratory testing of samples from enterovirus surveillance is also conducted. Among HFMD cases, 910 samples were received in 2011, 728 samples in 2012, and 25 samples in 2013. Of the 1663 HFMD samples collected from 2011 to 2013, 1061 (63.8%) NPEVs were detected by PCR. Among those NPEVs, 498 (46.9%) were EV71, 381 (35.9%) were coxsackievirus A viruses, 48 (4.5%) were unsubtypable, and 82 (5.1%) have pending sequence results. Two type 1 Sabin-like polioviruses were also detected from HFMD samples.

Cell sensitivity testing results are within acceptable range; however, polio type 1 in RD cells had a virus titre below the lower standard limit on 16 March 2012 testing. A new batch of cell lines was received on 16 April 2012, and since then, virus titre using RD cells has always been within the standard limit. All staff in the laboratory participated in the biosafety training course organized by NIHE using the WHO training modules.

One of the main challenges has been the implementation of real-time PCR technology for ITD of polioviruses. The laboratory requested support from WHO for acquisition of an ABI real-time PCR machine to initiate testing and carry out the implementation steps as per schedule: Step 1: 1 March to 15 April 2013; Step 2: 1 to 15 May 2013; and, when step 2 is successfully completed, Step 3: 1 to 15 June 2013.

(9) Viet Nam (Ho Chi Minh City)

Dr Nguyen Thi Thanh Thao from the Pasteur Institute in Ho Chi Minh City presented the surveillance activities in southern Viet Nam. The institute’s Laboratory of Enteroviruses is responsible for testing samples from AFP surveillance in southern Viet Nam. Stool samples were received from 189 AFP cases in 2011 and 291 in 2012. Polioviruses were isolated and two VDPV2 were detected in 2012. Among NPEVs detected in 2011 and 2012, 29 were EV71 and
27 were echoviruses, coxsackievirus A, coxsackievirus B, EV75 and EV79. Samples were collected from contacts in areas where the VDPV patients live. The test results showed that all 29 samples were negative. During this period, the laboratory also tested samples from children with HFMD or central nervous system infections by PCR. A total of 3583 samples from enterovirus surveillance were tested. Among these samples, 2985 (83.3%) NPEVs were detected, and out of those NPEVs, 2209 (74.0%) were EV71 and 776 (26.0%) were other NPEVs, and no poliovirus was isolated.

Cells were evaluated for sensitivity to all three poliovirus serotypes midway through their use of 15 passages. The results were within the expected titre and were reported to WHO regional laboratory coordinator within 48 hours. Step 1 of the implementation plan for real-time PCR was scheduled for February and March 2013, at which time training for staff in the laboratory will also be conducted.

Biosafety training for all the staff in the laboratory was conducted in 2012 using the WHO training modules. All laboratory staff are required to attend a biosafety training course conducted by the institute before working in the laboratory.

2.1.5 Laboratory quality assurance

(1) Report on proficiency testing: virus isolation

Dr Bruce Thorley from the WHO polio RRL in VIDRL, Australia reported on the results of the second WHO virus isolation PT done in 2011 using the new virus isolation algorithm. As he presented the method used to score the PT results. it was explained that 20 points were deducted from the score when polio-negative samples were contaminated with poliovirus or when poliovirus was missed, 5 points were deducted when any sample was contaminated with NPEV or other enteric virus, up to 10% was deducted when the interpretation of worksheets or use of the algorithm was incorrect, and 5% was deducted for each week of delayed reporting. A passing score was 90%. 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. All laboratories identified low titre poliovirus in Sample 6, and one laboratory observed cytopathic effect in RD only. Some laboratories maintained RD-RD & L20B-L20B in addition to new algorithm RD-L20B-RD & L20B-RD.

VIDRL received the 2012 PT late in the year, and one laboratory is still completing the test. However, some issues associated with the 2012 PT were discussed: (1) observations stopped on Day 9 (five days primary inoculation and four days passage); (2) readings of uninoculated cell culture controls were not provided; and (3) cell line passage was not indicated; e.g. L20B 21-5. No extra marks were given for additional testing by PCR and the results of PT should be based on cell culture observations.

(2) Reporting on ITD proficiency testing

Dr Olen Kew from the Division of Viral Diseases in US CDC discussed proficiency testing for polio molecular diagnostic methods. He said that PTs are conducted to assess proficiency of GPLN ITD laboratories in probe hybridization, conventional diagnostic PCR and real-time RT-PCR. They are also used to field-test the reliability and durability of polio molecular reagents and to help identify GPLN training needs. The US CDC-supported polio molecular assays are: nucleic acid probe hybridization NAPH for ITD (VP1 probes for each of
the three Sabin strains); diagnostic PCR for ITD (conventional PCR and real-time RT-PCR); and VDPV VP1 real-time RT-PCR. PT panels for molecular assays are composed of 10 unknowns (lyophilized non-infectious RNAs) and generally include polio-positive samples (singles and mixtures), NPEVs and negative samples. Laboratories are required to send their results and raw data to US CDC within seven days. A total score of $\geq 90\%$ out of 100$\%$ is needed to pass the PT. Fifty per cent of the total score is based on final results, and 50$\%$ is based on correct data interpretation. A 5$\%$ deduction is imposed for each week of late reporting. All polio network laboratories in the WHO Western Pacific Region performed the 2012 PT using real-time RT-PCR ITD and real-time RT-PCR VDPV except for polio laboratories in Hong Kong (China) and New Zealand, which used conventional PCR. It was concluded that all laboratories in the Western Pacific Region that participated in the 2012 PT are highly proficient in polio PCR assays, and the turnaround time for results is excellent.

(3) Laboratory quality assurance of China polio laboratory network: introduction of new algorithm

Dr Yong Zhang from NPL at China CDC presented on laboratory quality assurance of China’s polio laboratory network. The stool samples collected from AFP cases increased in 2011 (12,253) and 2012 (12,227) compared in 2010 (10,431), and the percentage of AFP cases with adequate stool specimens was 91$\%$ during the period 2011–2012. The NPEV isolation rate was about 12$\%$ in 2012, and the poliovirus isolation rate was 2$\%$. All laboratories scored 100$\%$ on the virus isolation PT in 2010 and 2011. In September 2012, the NPL and 23 provincial polio laboratories performed the ITD and VDPV screening PT provided by US CDC and WHO. All laboratories except Guizhou provincial polio laboratory passed the 2012 PT with a 100$\%$ score. The NPL performed the VP1 sequencing PT provided by US CDC and WHO in December 2012 and passed the PT with 100$\%$ score. On-site accreditation review was conducted by WHO staff in 13 provincial polio laboratories in China in 2011, while 11 provincial polio laboratories in China were reviewed in 2012.

A new cell sensitivity standard for provincial polio laboratories was established: L20B (passage 21 [P21]) and RD-A (P229) cells with low passage were sent to the 31 provincial polio laboratories, which also established a new cell bank of the two cell lines. Cell sensitivity results of the NPL and provincial polio laboratories were sent to the WHO Regional Office for the Western Pacific within 48 hours. Thirty provinces are reporting cell sensitivity test results to the NPL regularly, and all of the results are within acceptable range. The Tibet polio laboratory is re-establishing the LQC standard and cell bank. It is noted that the supply of liquid nitrogen is not on time or regular. Mycoplasma testing was performed on the cells and fetal bovine serum, and the results were negative.

In 2012, three training courses were conducted: (1) an annual workshop of China’s polio laboratory network was held in Yunnan province in February and the performance of new algorithm was highlighted; (2) two rounds of the training course on real-time PCR were held in Beijing on 5–16 March 2012, with two experts from US CDC invited as resource persons; and (3) the fourth training course on environmental surveillance was held in Beijing on 20–22 December 2012. With the introduction of a new algorithm for virus isolation, quality indicators have changed; hence, maintaining high-quality performance is a big challenge for China polio LabNet.
(4) Monitoring of cell sensitivity testing

Dr Fem Julia Paladin, Technical Officer (Laboratory), WHO Regional Office for the Western Pacific, reported on implementation of cell sensitivity testing in the Western Pacific Region from August 2011 to February 2013. Recommendations on cell sensitivity testing made during the 2011 VPD laboratory networks meeting were reviewed. 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, with more laboratories following the recommended strategy for monitoring the sensitivity of cells at appropriate passage levels, analysing the data using the trend chart, and reporting results within 48 hours to the regional laboratory coordinator. The recommended documentation for reporting cell sensitivity test results is yet to be implemented by China’s polio laboratory network. Performance issues were resolved through close communication with the regional laboratory coordinator and implementing cell sensitivity/cell culture recommendations during accreditation reviews. However, it is important to analyse and correlate cell sensitivity testing results with NPEV isolation rates and field conditions. Network laboratories, including China provincial laboratories, are encouraged to continue testing and reporting results, including absolute passage numbers of cells, titration data and trend charts, in a timely manner.

(5) Accreditation of ITD and sequencing laboratories

(a) WHO poliovirus sequencing PT update

Dr Olen Martin Kew from the Division of Viral Diseases, US CDC presented an update on the WHO poliovirus sequencing PT. A pilot sequencing PT was conducted from June to December 2011. The same set of RNAs was used as the ITD RT-PCR PT panel and VP1 sequencing PT panel. Individual Sabin samples were sequenced but not wild RNAs. Sequencing virus mixtures was optional and timeliness of reporting was not stipulated as a requirement. Participation in the pilot PT was required, but the score was not considered for accreditation. Seventeen laboratories from 15 countries participated in the pilot sequencing PT, including five laboratories from countries and areas the Western Pacific Region: Australia, China, Hong Kong (China), Japan and Singapore. All 17 laboratories submitted their results, 15 laboratories obtained sequences that were identical to the expected sequence of the PT panel, and two laboratories in the Western Pacific Region submitted partial results. Individual feedback was provided to each laboratory.

The 2012 sequencing PT was distributed to laboratories in November 2012. The requirements for reporting the results were established, and the timeline for reporting the results was within 14 days upon receipt of the PT panel. Submission of complete documentation, gel images, raw data, edited and final consensus sequences were required. Sequencing virus mixtures was optional. Primers were shipped with the PT samples to all laboratories. Scoring of the PT was discussed. Panels for ITD RT-PCR and sequencing were prepared separately because the ITD panel included too many virus mixtures. Twenty-one laboratories participated in the PT. Sixteen laboratories passed, and two laboratories did not pass due to problems in sequencing two of the samples (wild poliovirus RNA). Three laboratories have not yet reported results because of delays in distributing the panel (due to country importation requirements). A 90% score is required for passing the PT. The test is based on a score of 100 points that are broken down as follows: 10 points for documentation; 10 points for timeliness; and 20 points for each of the four samples (further broken down as amplification, 2.5 points; primer selection, 5 points; editing, 2.5 points; and sequencing, 10 points), for a total of 80 points. It was mentioned that the 2013
sequencing PT panels will be distributed soon and grading will be more stringent. Also, virus mixtures must be sequenced. Double-stranded sequence will be required throughout VP1. The next steps for PT of the sequencing laboratories were outlined: (1) US CDC will prepare probably one combined panel for ITD and sequencing; (2) USCDC will prepare sequencing kits; (3) WHO will continue to define the process for establishing and adding new sequencing laboratories by region; and (4) WHO will conduct additional accreditation visits and will ask laboratories to self-administer the sequencing accreditation checklist.

(b) Accreditation of ITD and sequencing laboratories within GPLN

Dr Ousmane Diop from WHO Headquarters emphasized the crucial role of the laboratories in ensuring that the Global Polio Eradication Initiative meets its objectives. He then outlined the components of the external quality assurance programme that ensure laboratories are maintaining the highest standards. The components include coordinated staff training using standard curricula; standardization of procedures, whenever possible; procurement of reagents and supplies from validated and reliable sources; and proficiency testing, on-site accreditation review by external assessors and feedback to laboratory and stakeholders. While tracing the progress made by GPLN over the years, it was noted that the last two years have seen the greatest innovations, introduction of new technologies, expansion of the capacity of the laboratories to do real-time RT-PCR ITD and a remarkable impact of introducing the new algorithm of testing, which resulted in dramatic >50% reduction in time spent confirming wild poliovirus and VDPV. The polio laboratory manual, which is the guide for all 146 polio laboratories, will be updated in June 2013. In 2013, several polio laboratories of the Western Pacific Region will have real-time RT-PCR ITD function and approximately 20% of GPLN laboratories have ITD function.

He presented the accreditation criteria for virus isolation, ITD and sequencing laboratories within GPLN. He affirmed that sequencing polioviruses is the ultimate tool to confirm wild poliovirus and VDPV and this will guide the programme for shaping surveillance and/or outbreak responses. Accreditation of the polio laboratories is a means for monitoring the performance of GPLN. Currently, there are three levels of accreditation: accreditation of the national polio laboratory, regional reference laboratory and the regional sequencing laboratory. With more national laboratories taking on the ITD and sequencing functions, these levels of accreditation will be changed to “viral isolation laboratory”, “polio ITD laboratory” and “polio sequence laboratory”. WHO accreditation criteria for viral isolation, ITD and sequencing laboratories were presented. Accreditation outcomes include: (1) laboratory is fully accredited when all criteria are met; (2) laboratory is provisionally accredited when it passes PT but has other deficiencies; and (3) laboratory is non-accredited when it fails PT.

While explaining the PT protocol of GPLN, Dr Diop said that the annual preparation and distribution of PT panels were assigned to two GSLs: Rijksinstituut voor Volksgezondheid en Milieu (Bilthoven, the Netherlands) for viral isolation and US CDC for molecular tests. Progressive introduction of new technology in key laboratories and monitoring of performances were noted. A passing score for real-time RT-PCR and sequencing proficiency testing is >90%.

In summary, well-designed accreditation exercises assist to identify and resolve problems without punishing laboratories or their staff, ensure continuous improvement and sustainability of the network laboratory’s performance, promote relationships and standardize capacities within the network, and build confidence in the network. Accreditation exercises should be aligned with technology and methodology evolution, and should facilitate collection of information for
purposes of monitoring, planning and action. GPLN will also prospect links with other accreditation mechanisms such as Strengthening Laboratory Management Towards Accreditation (SLMTA), Strengthening Laboratory Quality Improvement Process Towards Accreditation (SLIPTA), and International Organization for Standardization (ISO) 15189.

2.1.6 Video presentation for use of FTA card

Participants were shown a training video on the use of the Fast Technology for Analysis of nucleic acids (FTA) card. The video can be used to train laboratory staff on the proper techniques and processes for applying samples to FTA micro cards in order to ship samples safely to a reference laboratory for testing. The video described standard operating procedures associated with supplies and materials, personal protective equipment, biosafety cabinets, and other related equipment. Biosafety practices were emphasized since laboratory staff will be working with live viruses. It was explained that after applying a sample onto an FTA card, the card is allowed to dry inside the biosafety cabinet for about one hour at room temperature; the sample should not be heated. FTA cards with air-dried samples should be placed in clean, dry ziplock bags and stored in a refrigerator set at 4 °C or in a freezer at -20 °C. For shipment, cards should be placed in a regular shipping container. Extraction of RNA from FTA cards with air-dried samples was also presented. It was emphasized that FTA cards are useful when shipment of isolates is difficult or regulated.

2.1.7 Detection of poliovirus from non-AFP specimens and environmental surveillance

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

Dr Yong Zhang from China CDC presented on the progress of environmental surveillance in China. Three training workshops on environmental surveillance were conducted from 2007 to 2010, and a fourth training course on environmental surveillance was held in Beijing on 20-22 December 2012. Environmental surveillance was established to supplement AFP surveillance activities, to predict enterovirus-associated diseases and to detect emerging or re-emerging pathogens in China. Wastewater samples are collected and processed using MgCl2 concentration method. Continuous surveillance is being conducted in Shandong (Jinan City and Linyi City). A total of 187 polioviruses were isolated during the period 2008–2012. All polioviruses were identified as vaccine-like, except one that was identified as VDPV type 2. Several human enterovirus (HEV) types were detected from the environmental samples, with the predominant types being E6, E7, E11 and E12. Environmental surveillance was also conducted in Guangdong (Guangzhou City), where HEV-type distribution was similar to that of Shandong, i.e. E6, E7, E11 and E12. After an OPV campaign in early 2011, polioviruses were isolated from the environmental samples.

In conclusion, 10 provinces joined the environmental surveillance project in China in 2012. It is important to keep regular and long-term surveillance. HEV-A, HEV-B and HEV-C have been isolated from sewage samples since 2008. Also, virus isolation from both environmental and clinical sources could be complementary assessment approaches to trace prevalent and silent circulating enteroviruses in the human population.

(2) Australia experience

Dr Bruce Thorley from VIDRL presented on the detection of poliovirus in non-AFP samples. In Australia, IPV has been used since November 2005, and environmental surveillance was initiated in 2010. From 2010–2012, a total 36 samples were collected and no poliovirus was detected. NPEVs were isolated by cell culture from 81% of samples (29 NPEVs out of 36 samples) and in 94% of samples by pan EV RT-PCR (34 NPEVs out of 36 samples). E3 was isolated from stool of an AFP case in Newcastle in 2010, and found clustered with viruses isolated from sewage in Armidale and Newcastle in the same year. The Australian Government will support environmental surveillance testing for three more years (2012–2015). The importance of implementing environmental surveillance was emphasized. The testing protocol of environmental samples, which adopted the WHO protocol established by the polio laboratory in Finland, was described. 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 HEV in concentrate by in-house pan-enterovirus semi-nested RT-PCR (panEV snRT-PCR), and (2) HEV identification from isolates (cell culture) followed by CODEHOP RT-PCR. There is a good correlation of results between environmental surveillance and other surveillance systems.

(3) Malaysia experience

Mohd Apandi bin Yusof from IMR presented on environmental surveillance in Malaysia. He noted that AFP surveillance and immunization coverage were generally satisfactory in Malaysia; however, there were states/areas with poor performance for AFP surveillance indicators. Environmental surveillance for polio was initiated to identify reservoir communities and to target them with an immunization campaign to boost the herd immunity. Mr Apandi bin Yusof discussed the criteria used to select the three sites for environmental surveillance, namely, the Federal Territory of Kuala Lumpur, Selangor and Sabah. Activities carried out to implement the surveillance were also presented. The testing protocol of environmental samples, which adopted the WHO protocol established by the polio laboratory in Finland, was described. The cell culture method is used to identify HEV, followed by ITD (polio) and sequencing (NPEV). From January to December 2013, 13 Sabin-like polioviruses (six PV2 and seven PV3) and 23 NPEVs were detected from 36 sewage samples. NPEV types were: E7 (n=6), E6 (n=3), E30 (n=1), E11 (n=1), E5 (n=1), CB4 (n=7), and CB5 (n=4).

2.1.8 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 Tan Xiaojuan from China CDC presented on HFMD surveillance, epidemiology and pathogenic spectrum and molecular epidemiology of enterovirus 71 (EV71) and coxsackievirus A16 (CVA16) in China. EV 71 and CVA16 are the major pathogens of HFMD. 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 2007, a large-scale outbreak of HFMD associated with EV71 occurred in Linyi city and Shandong province, with 1149 cases reported, including 11 severe cases and 3 fatal cases. An outbreak of unknown viral infection in Fuyang city of Anhui province in March and April 2008, which was later identified as an EV71 outbreak, became a nationwide HFMD epidemic. Since
2008, a nationwide HFMD outbreak has occurred every year in Mainland China, becoming one of the most serious threats to young children. HFMD was added to the National Notifiable Disease Report System as the 38th legally notifiable disease on 2 May 2008.

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 in the HFMD LabNet were virus isolation, detection of RNA (RT-PCR and real-time RT-PCR for EV71, CA16 and other enteroviruses), serological method as well as sequencing for typing. In 2008 and 2009, the laboratory results showed that EV71 accounted for >80% of severe HFMD cases and >92% of fatal HFMD cases. In 1995, EV71 was isolated for the first time from the blister of an adult, which belonged to the C genotype. Genotyping of EV71 based on VP1 gene showed that the EV71 isolate belonged to the C3 subgenotype in 1997, and subgenotype C4 strains have predominated since 1998. One B5 subgenotype was detected from Fujian in 2009. From 1998 to 2009, subgenotypes C4 (clade C4a and clade C4b) evolved due to their circulation for 11 years in mainland China. Subgenotype C4 clade transition happened from C4b to C4a between 2002 and 2004. The phylogenetic analysis of the complete genome of EV71 detected from 1998 to 2009 showed that there might have been recombination events between EV71 and CA16 strains that were persistently circulated in China for at least 14 years. Evolutionary analysis of subgenotype C4 in China showed that the virus evolved during 1994 and was maintained at a constant and low level before 2007, but exponential growth from 2007 to 2008 was consistent with the large-scale outbreak of HFMD. Subgenotype C4 is still at a high level currently, which will be a big challenge for HFMD control and prevention in the coming years in China. Coxsackievirus A16 is less often associated with severe or fatal cases of HFMD; therefore, analysis of the virus is seldom performed. Nonetheless, analysis of the molecular evidence of persistent epidemic and evolution of subgenotype B1 coxsackievirus A16 associated with HFMD in China showed that B1a and B1b have been circulating in China since 2002. There is no evidence showing the evolution of subgenotype B1 coxsackievirus A16 in China.

(2) Republic of Korea experience

Dr Doo-Sung Cheon from Korea CDC presented on HFMD and other enterovirus surveillance in Korea. Enterovirus Laboratory Surveillance Networks was established in 2006 under the NPL. There are seven public regional institutes and 38 surveillance hospitals involved in HFMD surveillance system. Nearly 2,000 enterovirus cases are reported each year and most cases came from Seoul, Busan, Kyeonggi, and Gwangju regions. The methods used for the diagnosis of enterovirus are cell culture, real-time RT-PCR and GeneXpert and confirmed by RT-PCR (VP1) and genotyping. With the use of real-time RT-PCR since 2008, the positive rate of enteroviruses increased to 39.2% from 26.4% using VP1 nested RT PCR in the previous years. There was a major outbreak of EV71 infection in 2009 and 2012, and three patients have died with EV71 infection. Laboratory data showed that in 2008, prevalence of CVA10 (n=18, 34.0%) and E3-E33 (n=19, 33.9%) was noted, but EV71 was not detected. In contrast, in 2009, prevalence of EV71 (n=91, 55.2%) and E3-E33 (n=10, 6.1%) was predominant, but CVA10 was not detected. Other genotypes such as CVA12, CVA16, CVA2-CA6, CVB1 and CVB3 are also dominant in the Republic of Korea.

Enterovirus genotype data from 2010 to 2012 showed that EV71 was the major pathogen causing HFMD with neurological complications, comprising 78.1% of the total enteroviruses detected. On the contrary, viral meningitis is caused by coxsackievirus B consisting of about 55% of the total enteroviruses confirmed. The phylogenetic analysis based on VP1 gene of EV71 circulating in the Republic Korea during 2010–2012 showed that subgenotype C4a is in
circulation in the Republic of Korea. Diagnostic real-time PCR reagents that were developed specifically for EV-71 are rapid and sensitive, can detect 10–100 copies per reaction and can prevent carryover. The laboratory diagnostic flow for EV71 epidemic was revised in 2009 using real-time RT-PCR as the screening method. An EV71 vaccine project is under way.

(3) Japan experience

Dr Hiroyuki Shimizu from NIID reported on HFMD and infectious agent surveillance in Japan. Large-scale HFMD outbreaks with severe neurological diseases, including a number of fatal cases, occurred in the Western Pacific Region from the late 1990s. In 2008, a large HFMD outbreak with severe acute encephalitis mainly due to EV71 was reported in mainland China, and thereafter, a huge number of HFMD cases with severe neurological diseases have been reported every year in China. In 2012, more than 50 fatal cases were reported in Cambodia, and in Viet Nam, more than 200 fatal cases were reported from 2011 to 2012.

In the summer of 2011, a sudden increase in HFMD cases was reported to the National Epidemiologic Surveillance of Infectious Diseases System in Japan. The major causative agent of HFMD was CVA6 with very few severe cases. Typical clinical signs and symptoms of HFMD cases caused by CVA6 were fever; mild vesicles in oral mucosa; skin blisters on hands, arms, feet, legs and buttocks; and nail onychomadesis (periodic shedding of the nails). The prevalence of EV71 subgenogroups in the Asia Pacific region from the 1980s to 2012 was also discussed.

2.1.9 New polio laboratory database

(1) Update on standardization of AFP laboratory database

Mr Benjamin Bayutas, EPI Unit, WHO Regional Office for the Western Pacific presented updates on the new polio laboratory database. He discussed the current practice of reporting, whereby all laboratories submit data in Excel spreadsheets with varying formats at least once every month. China provincial laboratories submit data as part of the AFP surveillance dataset, in a comma-delimited text file format (*.CSV), while the China national laboratory submits data in MS Access database format (*.MDB). 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. 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 demonstrated the new polio laboratory database. The new standardized polio laboratory database includes core variables such as epidemiology and case information, virus isolation with options for poliovirus typing, ITD and sequencing, and 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. An updated draft version will be rolled out and implemented in 2013.
2.1.10 Biorisk management

Dr Bruce Thorley from VIDRL, Australia discussed biorisk management. A biorisk management training course is important because it promotes biosafety and biosecurity awareness in the GPLN. It can also be used to promote professional development, inform staff of recent developments, improve existing practices and instigate change to good management practices. A biorisk management advanced training course was conducted from 21 November to 1 December 2011 in Geneva. It was attended by participants from Argentina, Australia, China, the Republic of the Congo, India, Indonesia, the Russian Federation, South Africa, Syria and Tunisia, and was facilitated by staff from WHO and US CDC. Biorisk management was also introduced during the Second Regional Hands-on Training Course to Implement Polio rRT-PCR on 3–7 December 2012 at RITM, Philippines. Participants came from Hong Kong (China) (one participant), the Republic of Korea (one participant), New Zealand (one participant), the Philippines (two participants), and Viet Nam (two participants).

“Laboratory biosafety” is defined as containment principles, technologies and practices implemented to prevent unintentional exposure to pathogens and toxins, or their unintentional release, while “laboratory biosecurity” is the protection, control and accountability of valuable biological materials within laboratories in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release. The first step in identifying biological risks is to assess or identify all relevant hazards and threats. Information obtained through the assessment can then be used to determine and implement measures to mitigate the risks and reduce them to acceptable levels. “Biorisk management” is equivalent to the assessment, mitigation and performance (AMP) model. The hazards, risks and consequences of working in a laboratory with biological materials were enumerated. The biorisk assessment model (BioRAM) was also highlighted to provide visualization of the relative risks and help to identify risk measures. Participants were also shown how to use a risk matrix to identify the likelihood of a biological risk and to illustrate high-, moderate- and low-risk zones. Mitigation and control measures should consider the following hierarchy of control: elimination or substitution, engineering controls, administrative controls, practices and procedures, and personal protective equipment. The process of setting and reviewing objectives and implementing programmes to achieve them provides a mechanism for the organization to continually improve its biorisk management system and to improve its biorisk performance. Performance includes control (processes, procedures, structures and responsibilities to manage biorisk), assurance (systematic process of checking the system through audits and inspections), and improvement (setting and achieving biorisk goals based on internal and external feedback).

Laboratory biorisk management is a system or process to control safety and security risk associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities, and is based on CWA 15793:2011 standards, defined by the European Committee for Standardization Workshop Agreement in 2011. The risk assessment strategy was also emphasized.

2.1.11 New vaccine laboratory networks

Dr Fem Julia Paladin, WHO regional coordinator for new VPDs, presented on the establishment of new vaccine laboratory networks. Prior to 2009, surveillance for rotavirus and invasive bacterial vaccine-preventable disease (IB VPD) was supported by various organizations and donors as time-limited projects. In mid-2009, WHO shifted its funding (through the Global Alliance and partners) and support to building sustainable laboratory networks through country
ownership and integration with existing networks and surveillance systems, standardized surveillance and laboratory procedures, and collection and reporting of standardized data. The global and regional rotavirus and IB-VPD laboratory networks were established in October 2010 with defined roles and responsibilities, those being: (1) estimate disease burden due to rotavirus and pathogens causing IB VPD; (2) monitor circulation of specific serotypes/serogroups of IB-VPD pathogens and rotavirus genotypes and changes in distribution and anti-microbial susceptibility; and (3) detect outbreaks or epidemics early. The structure of the new VPD laboratory networks in the Western Pacific Region, which follows the polio and measles laboratory networks, consists of in-country laboratories with ongoing rotavirus and invasive bacterial disease surveillance and RRLs with expertise in gastroenteritis viruses and invasive bacterial pathogens.

The IB VPD laboratory network in the Western Pacific Region comprises two RRLs, one in the Republic of Korea (Korea CDC) and another in Melbourne, Australia (Microbiological Diagnostic Unit Public Health Laboratory [MDU PHL]), and four national laboratories in Mongolia (National Center for Communicable Diseases), the Philippines (RITM) and Viet Nam (NIHE and Pasteur Institute). Cambodia (National Institute of Public Health) and Papua New Guinea (Port Moresby General Hospital) store cerebrospinal fluid (CSF) specimens for shipment and testing to Korea CDC and MDU PHL in Australia, respectively. To build capacity of IB-VPD network laboratories in the Western Pacific Region, a series of training courses attended by staff was conducted in 2011 and 2012; equipment, reagents and test kits were also provided. A quality assurance programme is being established. The 2011–2012 IB-VPV surveillance data showed that across countries, 2–26% of suspected meningitis cases are VPDs. Frequent challenges in the global and regional IB VPD laboratory networks include the following: low bacteria isolation and detection; insufficient volume of CSF for PCR testing, thus, low serotype data by PCR; transport logistics (import permits for referral to RRL); poor communication and high attrition of staff at sentinel sites; and data quality and management.

The Western Pacific Region’s rotavirus laboratory network comprises three RRLs (China CDC, Korea CDC and Murdoch Childrens Research Institute at the Royal Childrens Hospital, Melbourne, Australia), eight national laboratories (Cambodia, Fiji, the Lao People’s Democratic Republic, Mongolia, Papua New Guinea, the Philippines, northern and southern Viet Nam), and 18 subnational laboratories (17 provincial laboratories in China and one in Papua New Guinea). Laboratory procedures for testing stool samples are rotavirus antigen detection by enzyme-linked immunosorbent assay (ELISA) and standardized genotyping methods following the Murdoch Childrens Research Institute’s protocol. To build capacity in rotavirus detection and strain characterization, training was conducted for staff of network laboratories in the Western Pacific Region in 2011 and 2012. A quality assurance programme for the rotavirus laboratory network in the Western Pacific Region is being set up. Some of the issues of concern were discussed, including monitoring of ELISA performance in the quality control chart, delayed referral of specimens to RRL for genotyping and quality control, and completion of standardized rotavirus RRL and national laboratory database roll-out.

Since the laboratory networks are new, modifications in work practices are needed to meet required performance indicators and to implement quality assurance mechanisms and biosafety requirements. Monitoring of laboratory performance, use of standardized laboratory data reporting, supportive on-site assessment and supply management are critical to effective coordination of the new laboratory network activities.
2.1.12 Hepatitis B control initiative in the Western Pacific Region

(1) Hepatitis B control goals

Dr Karen Henessy, Technical Officer, WHO Regional Office for the Western Pacific, presented on the hepatitis B control goals in the Western Pacific Region. In 2005, Member States adopted two hepatitis B control targets, specifically, a milestone to reduce chronic hepatitis B prevalence to less than 2% in children under five years old by 2012, and the goal to reduce prevalence of hepatitis B infection to less than 1% among children under five years of age; the target year to meet this goal is yet to be established. Three tactics are being used to achieve the goal: ensure high vaccination coverage, measure impact by serosurveys, and verify achievement of goals. The immunization coverage criteria set for achieving the goal of less than 2% prevalence is more than 65% timely birth-dose coverage, i.e. vaccine given within 24 hours of birth, and more than 85% coverage with three doses of hepatitis B vaccine (HepB3). Data from priority countries in 2008–2011 showed that Cambodia and Viet Nam have met the target of HepB3 coverage, while the Lao People's Democratic Republic, Papua New Guinea (though had met the target in 2010) and the Philippines have not met the target. For timely birth-dose coverage, only Cambodia has met the target of more than 65% coverage in 2011.

Serosurveys are recommended because hepatitis B infection in children rarely shows symptoms and delayed disease outcomes and disease surveillance is not ideal approach for monitoring impact. Because laboratory quality assurance is very important, there should be standard laboratory procedures including proper quality control and assurance. Rapid tests are acceptable when laboratory capacity is limited or resource settings are constrained. A comparison between ELISA and rapid tests showed that ELISA has high sensitivity and specificity.

Verification is crucial to ensure a standard and independent process for measuring achievements towards the regional goal. The verification process was discussed. The Regional EPI Technical Advisory Group (TAG) and Hepatitis B Expert Resource Panel have recommended setting 2017 as the target year to achieve the goal of reducing the prevalence of hepatitis B infection to 1% in children at least five years of age. Support will be given to countries to increase routine and birth-dose coverage. Collaboration with maternal and child health programmes to increase access to newborn care and vaccination will be emphasized. For laboratories, guidance to countries on acceptable assays and rapid tests will be updated, including proper quality control and assurance. Laboratory criteria in the verification process need to be improved as needed.

(2) Hepatitis B regional reference laboratory

Dr Michael Catton, Director, VIDRL, Australia discussed the role of the RRL for hepatitis B. In 2010, VIDRL was designated as a RRL for hepatitis B following the endorsement of a World Health Assembly resolution on viral hepatitis. Currently, laboratory staff are contributing to Hepatitis B Expert Resource Panel in the Western Pacific Region, as well as global consultations. Most notably, VIDRL has developed and validated a novel phenotypic assay for hepatitis B virus (HBV) variants that detects all HBV genotypes. This BioPlex multiplex assay detects the hepatitis B surface antigen (HBsAg) vaccine-escape mutant, sG145R, by phenotype. There is potential for the assay to be used as a high throughput phenotyping platform for HBsAg to monitor for the vaccine-escape mutant. A plan to develop a hepatitis B surface antibody (anti-
HBs) assay to identify vaccine-escape anti-HBs profiles will be initiated. Vaccine-escape mutants have the potential to endanger the hepatitis B immunization programme.

The molecular epidemiology of HBV in indigenous Australians is unknown. Seroprevalence rates approach endemicity, with some studies suggesting seroprevalence rates between 3.7% and 14.8%. Indigenous Australians have had universal vaccination since 1988; however, some small studies have raised concerns over long-term immunity. A study of HBV in this population, therefore, would have public health significance regarding vaccine efficacy. High rates of hepatocellular cancer have also been reported for indigenous populations, the incidence of hepatocellular cancer is 4.4 times higher in indigenous Australians compared to non-indigenous Australians in the Northern Territory, with the most common etiological factor being HBV. A chronic hepatitis B antiepadnaviral resistance mutation (CHARM) study is being conducted from across the top end of the Northern Territories. This study aims to describe viral mutation profiles in patients diagnosed with chronic hepatitis B receiving antiepadnaviral therapy. Mutation analysis detected the classic vaccine-escape mutant, sG145R, in two out of 46 samples (4%). No antiviral resistance mutations were detected. The laboratory was also able to amplify 44 sequences covering the polymerase/surface antigen region, and the phylogenetic analysis showed that each of the virus sequences obtained from indigenous Australians belonged to a single subgenotype of C, known as C4. The isolated genotype C4 HBV represents a novel strain, subtype ayw3, which may be a result of recombination.

Another research project was conducted to determine the genotypes of HBV among Torres Strait Islanders, and also to explore the level of knowledge about HBV in infected people, including information on prevention and treatment. Serum samples were sent to VIDRL for HBV genotyping. The HBVs isolated from the Torres Strait Island population are potentially new subgenotypes (C1) of genotype C. Eighteen of 40 samples received were PCR positive, and sequencing of the conserved polymerase domains and basal core promoter/precore (BCP/PC) region was performed. The sequence results showed the G145R vaccine-escape mutation was detected in one sample and BCP/PC mutations were detected in 12 out of 17 samples (70%). The prevalent HBV genotype in indigenous Australians is genotype C. This discovery has implications for public health, as genotype C is the most oncogenic of HBV genotypes associated with increased risk of progression to liver disease and hepatocellular cancer compared to all the other HBV genotypes. Significance of those novel strains requires further exploration.

2.2 Measles and Rubella Laboratory Network

Opening session

Dr Youngmee Jee, Regional EPI Laboratory Coordinator, welcomed all participants and advisers. Dr Sergey Diorditsa, EPI Team Leader, opened the meeting. He acknowledged the contribution of the laboratory networks 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.

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

(1) Global and regional updates on eliminating measles and rubella

Dr William Schlutter, Medical Officer from the WHO Regional Office for the Western Pacific, made a presentation on global and regional updates on measles and rubella control. It
was noted that as the immunization coverage with one dose of measles-containing vaccine (MCV1) improved from just over 10% in the 1980s to over 90% in 2011, the number of reported cases globally declined from over four million in 1980 to 400,000 in 2011. Intensified immunization campaigns since 2001 and introduction of the second dose of measles-containing vaccine (MCV2) in the routine immunization schedule by most countries has contributed to the effective control of measles. Except for the South-East Asia Region, the other five WHO regions have now established regional measles elimination goals.

Dr. Schluter outlined the measles elimination goal and the status of measles elimination in the WHO Western Pacific Region. The Regional Committee for the Western Pacific established 2012 as the target year for measles elimination when it endorsed resolution WPR/RC56.R8 in September 2005. In October 2005, Regional Committee resolution WPR/RC61.R7 called on Member States to accelerate the measles elimination goal and to accelerate the control of rubella and prevention of congenital rubella syndrome (CRS). Commitment to eliminate measles and control rubella was once again reaffirmed with resolution WPR/RC63.R5 in September 2012. It was noted that countries in the Region have already achieved >95% national MCV1 coverage and >91% national MCV2 coverage. There has been tremendous progress in measles control since 2008, and the major reduction in number of confirmed cases in the Region was due to SIAs conducted in China.

In 2011, throughout the Region, over 94 million doses of measles-containing vaccine were given through routine immunization services. It was noted that while most countries of the Region have achieved MCV1 coverage of over 95%, there are three countries that have just 50% to 78% MCV1 coverage. Even among countries that have achieved national MCV1 coverage of over 90%, there are areas with suboptimal coverage of less than 70%. Seven countries of the Region have achieved over 95% MCV2 coverage, and most other countries have achieved between 80% and 94% coverage, but there are still three countries (Lao People's Democratic Republic, Solomon Islands and Vanuatu) that have not introduced MCV2 in the routine immunization schedule. A series of SIAs were conducted between 2010 and 2012 in 10 countries. Measles incidence in the Region dropped from 82 per million in 2008 to 27 per million in 2010, and 5.9 per million in 2012. There was also an improvement in the performance indicators towards measles elimination in the Western Pacific Region from 2007 to 2012.

Dr. Schluter discussed the ongoing measles transmission in Malaysia and the Philippines. The Malaysia outbreak started in 2011 (n=1573 cases) and continued in 2012 (n=2112 cases); by end of 2012, the outbreak had been controlled. The vast majority of measles cases were children under five years of age. The Philippines measles outbreak started in 2010 (n=6363 cases) and continued in 2011 (n=6519 cases). The number of measles cases dropped in 2012, with only 1430 cases reported. The age distribution of measles cases in the Philippines is bimodal, meaning, some cases were very young children and a considerable number of cases were young adults and adolescents.

Dr. Schluter also outlined the significant progress in rubella control globally since 1996. In 1996, only 83 countries comprising 13% of the birth cohort were using rubella vaccine, whereas in 2011, 130 countries comprising 41% of the birth cohort were using rubella vaccine in their national immunization system. Since 2000, there has been steady growth in the number of countries in the Western Pacific Region offering rubella vaccine in routine immunization programme. The reported CRS incidence in the Western Pacific Region was higher (n=201) than any other WHO region in 2011 since there was a specific CRS investigation conducted in Viet Nam. In 1996, it was estimated that 120,000 infants were born with CRS globally. In 2008,
the estimate was approximately 112 000, which is a decrease that has occurred in the presence of increasing birth cohort size. The impact of the rubella elimination activities is clearly seen in the Region of the Americas and the European Region.

In the Western Pacific Region, two countries (Lao People's Democratic Republic and Solomon Islands) will be conducting measles and rubella immunization campaigns and will introduce rubella-containing vaccine (RCV) in routine immunization in 2013. Three countries (Cambodia, Vanuatu and Viet Nam) have plans to conduct measles and rubella immunization campaigns in 2013. Papua New Guinea is conducting disease-burden studies with possible introduction of RCV in the next few years. Rubella surveillance is not established as well as measles surveillance; thus, for countries that do not classify suspected rubella cases, classifications are being made based on rubella IgM results from national surveillance and laboratory reports. Surveillance is improving steadily, but challenges remain. Case detection rates are still below the recommended national level, while the proportion of cases that were clinically confirmed without laboratory testing is higher.

The next steps will involve the verification of measles elimination. The Regional Verification Commission will finalize the framework for verification during a meeting to be held on 18–22 March 2013.

(2) Update on the global measles and rubella laboratory network

Dr Miguel Norman Mulders, EPI, WHO Headquarters, presented an update of the WHO global measles and rubella laboratory network. Dr Mulders discussed firstly the global measles goals, which included Millennium Development Goal 4 (reducing deaths among children by two-thirds by 2015 compared to 1990), and also by 2015, meeting measles vaccination coverage targets of 90% at the national level and 80% in every district, lowering the reported incidence of measles to less than five cases per one million population, and reducing mortality due to measles by 95% (compared to 2000).

The Region of the Americas achieved measles elimination in 2010. The other WHO regions have set specific measles elimination goals as follows: Western Pacific Region by 2012, European Region and Eastern Mediterranean Region by 2015, and African Region by 2020. The South-East Asian Region has set a goal for measles mortality reduction by 95% by 2015 and will eventually set a goal for measles elimination. The goal set for measles mortality reduction by 95% by 2015 is being closely monitored. As of 2012, the Region of the Americas and the Western Pacific Region have achieved 100% and 90% reduction in measles mortality, respectively. The African Region, Eastern Mediterranean Region and European Region have achieved 84%, 62% and 45% reduction in measles mortality, respectively. The South-East Asian Region (excluding India) has achieved 70% reduction in measles mortality. India has achieved only 36% reduction in measles mortality; however, measles-related deaths will be dramatically reduced once all states have introduced MCV2 – 17 states are adding a second dose to the routine schedule, while 14 states are conducting immunization campaigns before introducing MCV2 into routine immunization system. It was noted that 141 countries have introduced MCV2 in the routine immunization schedule, and the 52 countries that have yet to introduce MCV2 in the routine schedule have implemented supplementary immunization activities covering all children. These efforts have led to 64% reduction in incidence of measles globally.

Towards achieving the goal of control of rubella and elimination of CRS, as of 2011, 131 countries covering 41% of the birth cohort have introduced rubella immunization in the routine
childhood immunization programme, compared to 83 countries covering 13% of the birth cohort in 1996. In comparing the total estimates of CRS, in 1996, it was estimated that 120 000 infants were born with CRS annually. In 2008, the estimate was approximately 112 000, which is a decrease that has occurred in the presence of increasing birth cohort size, meaning, there was a larger reduction in the incidence rate than was seen in the absolute numbers. As of 2011, around 67% of the Member States were reporting incidence of CRS. The greatest impacts of the rubella elimination activities were clearly seen in the Region of the Americas and the European Region. The Global Alliance for Vaccines and Immunization, in November 2011, began funding and supporting countries to use the rubella vaccine, in combination with measles vaccine, as part of catch-up campaigns targeting children between the ages of nine months and 14 years, 11 months.

Despite progress made, key challenges remain, most notably: importation of measles into measles-free regions (e.g. Region of the Americas), weak immunization systems, security risk limiting access to immunization, achieving and sustaining MCV2 coverage over 95%, decreasing the susceptibility gaps in the population including older age groups, and lack of human and financial resources to achieve the goals.

Dr Mulders acknowledged the important role of the WHO Global Measles and Rubella Laboratory Network in measles elimination and rubella control. Some of its responsibilities include confirming (or rejecting) clinical suspicion of measles or rubella cases, providing evidence for verification of elimination (including genotype surveillance), and monitoring the effectiveness of control/elimination efforts through SIAs or routine immunization. As of 2012, the Global Measles and Rubella Laboratory Network comprised 679 laboratories made up of GSLs, RRLs, and national and subnational laboratories. A total of 106 948 measles samples were tested globally and reported to WHO by the laboratory network in 2012. The European Region has the highest workload for measles (n=48 224) and rubella (n=34 472) testing. Of these samples, 30% were positive for measles and 23% were positive for rubella. In the Western Pacific Region, 15 883 samples were tested for measles and 13 116 samples were tested for rubella. Among the samples tested, 16% were positive for measles and 14% were positive for rubella.

A quality assurance programme is well implemented in the laboratory network. Results of the WHO global PT for accuracy of measles and rubella IgM detection in 2011 indicated that only one of the 209 laboratories that participated in the 2011 PT missed the 90% cut-off and most of the laboratories scored 100%. The results of the 2012 PT are being compiled and analysed. It was specifically noted that few laboratories were not able to receive the PT panel due to shipment issues. A molecular detection PT is being developed and will be rolled out as a pilot to a few laboratories and eventually on a global level.

Dr Mulders emphasized the power of molecular epidemiology as a tool in identifying pathways of transmission. It can identify the possible source of virus – indigenous or imported and vaccine derived or wild poliovirus – and monitor progress towards measles elimination. Baseline data of circulating measles and rubella virus genotypes are essential to determine indigenous viruses and to target control measures. Therefore, laboratories are required to submit genotype data to WHO-managed measles and rubella databases. From 1954 to 2012, sequence data for 14 165 measles viruses (24 genotypes) were submitted from 145 countries and areas to the measles nucleotide surveillance (MeaNS) database, and data on 1302 rubella viruses (nine genotypes plus four provisional) were submitted from 49 countries and areas to the rubella nucleotide surveillance (RubENS) database. Sequence data were submitted for 3000 measles and 400 rubella viruses in 2011 and for 1500 measles and 240 rubella viruses in 2012. As of 2012,
the measles genotypes in circulation were B3, D4, D8, D9, G3 and H1. The rubella genotypes in circulation were 1A, 1E, 1G and, most predominantly, 2B. While 113 Member States reported laboratory-confirmed rubella, only eight of them reported rubella genotypes. Laboratories were encouraged to submit more measles and rubella genotypes data. It was also reported that molecular testing capacity has become accessible to more laboratories. Specific training workshops focusing on molecular techniques are being conducted. Kit-based RT-PCR systems to facilitate use of the test are being developed. It was emphasized that oral fluid samples can be used for detection of measles and rubella RNA up to several weeks after disease onset.

Towards the elimination stage, particularly when incidence is zero, the focus and priority of the laboratory changes. Meeting elimination criteria for laboratory surveillance, especially timeliness of reporting results by laboratory within four days of receipt of sample, is a challenge. Also, more than 80% of laboratory-confirmed measles outbreaks should have adequate samples for virus characterization in a WHO-accredited laboratory. However, integration of the laboratory surveillance system into the national surveillance system (linking epidemiology-laboratory) should be improved. Identification of resources to sustain current capacity and meet the additional tasks remains a challenge.

(3) Update on the regional measles and rubella laboratory network

Dr Youngmee Jee, Regional Laboratory Coordinator from the WHO Regional Office for the Western Pacific, provided an update on the measles and rubella laboratory network in the Western Pacific Region. As of March 2013, 48 out of 50 functional network laboratories that sought WHO accreditation, including 30 provincial laboratories in China, had been accredited; the two remaining laboratories were the national laboratory in the Lao People’s Republic and Xinjiang subnational laboratory in China. Three additional subnational laboratories that have been designated include two subnational laboratories in Viet Nam and one subnational laboratory in Sabah Malaysia. The Brunei Darussalam laboratory is being considered to be a WHO national measles and rubella laboratory in 2013. In 2011, 18 out of 19 non-China laboratories scored 100% on the measles PT and one laboratory scored 95%; all 19 laboratories scored 100% for rubella. In 2012, based on the WHO ELISA PT, two laboratories did not meet one of the validity criteria and re-tested the PT samples. Completeness and timeliness of laboratory data reporting to WHO have improved, reaching 93% and 87%, respectively, in 2012, but timeliness of two laboratories was less than 80% (Papua New Guinea, 50% and Malaysia, 58%).

China shares measles and rubella genotype information with the WHO global laboratory network, and Japan shares the number of measles laboratory-confirmed cases in monthly case-based surveillance reports. Besides China, four other countries, namely, Cambodia, Malaysia, the Philippines and Viet Nam (south), processed the largest number of samples during 2010–2012 due to outbreaks. Dr Jee presented the impressive achievements made by three Mekong countries: Cambodia, the Lao People’s Democratic Republic and Viet Nam. In 2011, there were measles outbreaks impacting many provinces in Cambodia, and localized measles outbreaks in the Lao People’s Democratic Republic and Viet Nam. In 2012, no laboratory-confirmed or epi-linked measles case was detected in Cambodia, but one laboratory-confirmed case was reported in both the Lao People’s Democratic Republic and Viet Nam. There was still active circulation of measles in Malaysia, with 92% laboratory-confirmed in 2011 (1445/1569) and 79% in 2012 (1671/2112). In the Philippines, 49% of suspected measles cases were laboratory-confirmed in 2011 (3240/6555), and 41% in 2012 (593/1453). In early 2013, an outbreak of measles caused by the H1 genotype strain was confirmed in the Lao People’s Democratic Republic, reported from Phongsaly and Luang Namtha provinces.
Dr Jee outlined a rubella outbreak caused by the rubella 2B genotype in Fiji and other Pacific island countries in 2011–2012. 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. For quality assurance of the network laboratories, Dr Jee mentioned that confirmatory testing of a proportion of samples from national laboratories was performed in RRLs in Hong Kong (China) and Australia, and the concordance rates were over 90% for measles and rubella IgM detection by ELISA testing in 2012.

More network laboratories have been performing virus isolation and genotyping since the hands-on training in 2009, 2010 and 2012 in Hong Kong (China) RRL and in Beijing, China. The measles laboratories in Australia, 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 2011 and 2012 were B3, D4, D8, D9, D11, G3 and H1. Endemic genotypes in the Western Pacific Region include H1 (China, Lao People’s Democratic Republic, and Viet Nam) and D9 (Cambodia and Philippines). G3 strains were not detected in Japan, Malaysia and Singapore in 2012; however, the G3 strain was detected in Australia. Mixed genotypes related to importation were reported from Australia, Hong Kong (China) and Japan in 2011–2012: D4, D8, D9, G3 and H1 strains. After a hands-on training conducted in Beijing in July 2012 by China CDC, 30 out of 31 of the participating provincial laboratories provided results for the molecular practice PT. Following the training in Hong Kong (China) in October 2012, 12 out of 13 national measles/rubella laboratories that participated in the training provided results of the molecular practice PT. Most of the laboratories got excellent results. Genotype detection also improved in the Western Pacific Region, and among 119 cases related to importation in 2011, 68 cases (57%) had genotype information. The proportion of genotyped cases among laboratory-confirmed measles cases increased from 1.4% in 2010 to 8.0% in 2012. Yet, limited genotype data are available among laboratory-confirmed cases in the Philippines, and the laboratory confirmation rate is low in the Lao People’s Democratic Republic and Viet Nam. The Hong Kong (China) RRL plays a major role by conducting most of the confirmatory testing and genotyping in the Region.

Dr Jee enumerated remaining challenges: (1) low percentage of laboratory-confirmed cases among clinically confirmed cases in few countries; (2) low virus isolation samples collection rate; (3) discrepancy in the number of laboratory-confirmed measles cases in countries with non-WHO network laboratories involved in testing, namely: Australia, Japan, New Zealand and Singapore; (4) implementation of the WHO-recommended algorithm of testing by all laboratories; and (5) with the Region’s additional initiative to control rubella, laboratory confirmation for rubella and CRS cases should be emphasized.

Verification of measles elimination as a component of laboratory performance was also discussed. Verification of measles elimination for the Region is only possible when all countries are able to document interruption of endemic virus transmission for a period of more than 36 months. Therefore, it is essential that the laboratories fulfil the following criteria: (1) proportion of measles network laboratories that are WHO-accredited for serological and, if relevant, virological work (target: 100% of laboratories); (2) proportion of laboratories (government and private) that conduct measles diagnostic testing in the countries that have effective quality assurance in place (target: 100%); and (3) proportion of virus detection and genotyping results (where appropriate) completed within two months of receipt of specimen (target: ≥80% of specimens received).
Planned activities to further meet the elimination criteria include: (1) shorten timeliness of ELISA testing and reporting to four days from seven days; (2) increase laboratory confirmation and virus isolation/genotyping from both sporadic and outbreaks cases (both measles and rubella); (3) strengthen rubella and CRS testing and virus identification; (4) improve coordination in sharing of laboratory testing data among surveillance and laboratory programmes (non-WHO network laboratories); (5) network laboratories to initiate confirmatory testing in consultation with the Regional Office for the Western Pacific (Malaysia to send to Australia RRL), and high-workload laboratories (Philippines and Malaysia) to send two shipments instead of one; (6) network laboratories with molecular testing capacity to participate in measles rubella molecular PT (US CDC’s support) and WHO to increase funding for priority laboratories with molecular capacity in 2013; and (7) use oral fluid collection (Oracol) kit and serum samples collected in early stage (0–3 days) to strengthen molecular surveillance. Implementation of the recommendations from the last meeting in 2011 was reviewed and almost all recommendations were achieved.

2.2.2 Quality assurance of measles and rubella network laboratories in the Western Pacific Region

(1) Measles and rubella IgM proficiency test updates and confirmatory testing (Australia)

Ms Vicki Stambos from VIDRL presented an update on the measles and rubella IgM PT, the procedures for preparing WHO measles and rubella IgM PT panels, and an analysis of results of Panel 01106 from 211 network laboratories in all regions including the Western Pacific Region and Panel 01202 from laboratories in the Western Pacific Region excluding China’s provincial laboratories. For Panel 01106, 51 laboratories in the Western Pacific Region including 31 provincial laboratories in China participated and scored 100%, while 95% of the network laboratories in the Western Pacific Region that did Panel 01202 scored 100%. Network laboratories in the Western Pacific Region used a variety of diagnostic kits for doing PT: Beijing Beier, Bio-Rad, Denka Seiken, Haitai, Immuno-Biological Laboratories, Microimmune, Siemens, and Virion Serion IgM kits for measles IgM ELISA, and Euroimmun, Haitai, Immuno-Biological Laboratories, Kerunda, Siemens, and Virion Serion IgM kits for rubella IgM ELISA. One laboratory in the Western Pacific Region used an expired kit for testing. Besides coordinating the global PT annually, VIDRL also does the confirmatory testing of samples referred from Fiji, New Zealand, Papua New Guinea and the Republic of Korea. Results showed very good concordance rates. Laboratories were requested to contribute positive samples for the preparation of a PT panel.

(2) Confirmatory testing in Hong Kong (China)

Dr Jasmine Kwong, Medical and Health Officer from PHLC in Hong Kong (China), presented the results of confirmatory testing performed on clinical samples referred by nine national measles and rubella laboratories in eight countries. As the main confirmatory test, PHLC uses the Siemens indirect enzyme immunoassay (EIA) on measles and rubella IgM and also performs RT-PCR and genotyping on serum, nasopharyngeal, throat swabs, urine samples and culture isolates. As a reference laboratory, PHLC enforces quality assurance measures to ensure high-quality performance. Since 2007, serum specimens received annually for IgM confirmation have increased from 55 to 738. Specimens for RT-PCR have also increased from 10 to more than 100. Dr Kwong discussed the analysis of the results to determine the concordance rate between two laboratories. Results are considered concordant if they agree categorically, which means both the national laboratory and the RRL have reported the same positive/positive or
negative/negative results. When the equivocal result is reported as positive or negative, the optical density (OD) value of the test is considered. If the reported result does not tally with the result of the RRL, then it is considered discordant. Since 2009, all laboratories attained >90% concordance and four laboratories were consistently 100% concordant for measles IgM. The concordance rates varied from 81.5% to 100% for rubella IgM. When discordant results are found, the test is repeated to ensure it is repeatable. PHLC also liaises with the laboratory to find out the causes of the discordant results. It was emphasized that laboratories should provide information on date of rash onset and sample collection, and indicate whether the OD readings/cutoff (Siemens) have been corrected for better comparison.

Most of the measles viruses were genotype D9, while some were D8, D6, G3 and H1. Results of rubella genotyping revealed 2B as the most common genotype, but other genotypes detected were 1a, 1j and 1eE. The national laboratories were requested to provide a minimum of 200µl of serum (for both serology and molecular testing), and also consider sending swab or extracted RNA for genotyping.

(3) China Laboratory Network: confirmatory testing and proficiency test

Mr Mao Naiying, Associate Researcher from China CDC, presented the results of the proficiency and confirmatory testing in China’s measles network laboratories. All provincial laboratories received samples from VIDRL and scored 100% on the 2011 PT. Results from the 2012 WHO PT are still pending. For the 2011 PT, 15 laboratories used the Haitai measles IgM kit, 16 laboratories used the measles Verion Serion IgM kit, and one laboratory used the measles Beier IgM kit. Regarding rubella, 14 laboratories used the Haitai rubella IgM kit, 10 laboratories used the rubella Verion Serion IgM kit, and 8 laboratories used the rubella Kerunda IgM kit. China CDC performed detailed analysis of results received from the laboratories, and there were good correlations between kits used. However, some provincial laboratories got very high OD value of positive samples in both measles and rubella IgM testing.

For confirmatory testing, each provincial measles laboratory is required to send 30 serum samples which include 10 measles IgM antibody positive samples, 10 rubella IgM antibody positive samples and 10 negative samples to the national laboratory; however, some laboratories cannot provide the required number of serum samples due to insufficient number of measles cases. Results of confirmatory testing were provided to provincial laboratories within one month after the receipt of samples. In 2012, two provincial laboratories were not able to send samples for confirmatory testing, Hainan failed to provide a shipping permit for infectious substances and Tibet has no samples collected. The concordance rate of measles and rubella IgM detection was 100%. Some of the challenges mentioned were: only the strong positive samples were selected and sent to national laboratories for confirmatory testing, and some provinces cannot provide 10 measles IgM antibody positive serum samples yearly due to the decline in measles cases in China.

(4) Quality assurance programme for molecular techniques

Dr Paul Rota from US CDC presented the importance of quality control for molecular tests and the development of a protocol for molecular PT panels. He stressed that molecular characterization of measles and rubella viruses plays an increasingly important role in laboratory surveillance. US CDC provides validated protocols/kits for RT-PCR with low limit of detection, very high sensitivity and specificity; efficient real-time RT-PCR and multiple assay runs give consistent results. Dr Rota reported the results of the 2012 measles and rubella FTA card practice panels from four WHO regions. In the Western Pacific Region, 12 countries participated in the
measles real-time RT-PCR and genotyping RT-PCR practice panels, while 11 countries participated in the measles sequencing practice panel. For the rubella FTA practice panel, 11 countries participated in the real-time RT-PCR, genotyping RT-PCR and sequencing/genotyping practice panels.

In China, 30 laboratories – the national measles laboratory and 29 provincial laboratories – participated in the 2012 FTA practice panel for measles. All of them had standard genotyping RT-PCR results correct, including all controls. Fourteen laboratories correctly identified genotypes, while 16 laboratories sent PCR products to the national measles laboratory. All of the laboratories correctly identified positive samples by real time RT-PCR, but four laboratories incorrectly identified negative Sample 2 as a false positive. Dr Rota stated that all laboratories reported the correct results with minimal cross-contamination in standard, endpoint RT-PCR assays. The genotyping RT-PCR assays performed well and most laboratories correctly identified the genotypes. Results were reported in a timely manner. However, some problems were also encountered.

There will be an increased workload for RRLs to perform additional sequencing for national laboratories and increased workload at CDC to produce kits and quality control panels. The reporting format also needs to be improved. It was recommended that a take-home practice panel should be included as follow-up for all intercountry training courses focusing on molecular methods. Also, development of a standard method for molecular PT panel and feedback from all laboratories are needed. A revised standard protocol for a proficiency testing programme for molecular methods used in the WHO global measles and rubella laboratory network was presented. Successful completion of the molecular PT panel will be one of the criteria for WHO accreditation, along with evidence of implementation of routine quality control/quality assurance procedures for molecular methods.

2.2.3 Enhancing molecular surveillance

(1) Experience in Japan

Dr Katsuhiro Komase from NIID presented the two systems used to confirm measles cases in Japan: (1) measles ELISA testing, which is done mostly in commercial laboratories; and (2) RT-PCR, which is conducted in prefectural institutes of public health. Japan introduced a measles elimination plan and case-based reporting in 2008; the number of confirmed measles cases dropped from 11 007 in 2008 to 293 in 2012. Forty per cent of measles cases in 2011 and 32% in 2012 were confirmed by PCR. Measles genotype D5 strains, which used to be endemic in Japan, have not been detected since May 2010. In 2012, D8, D9, D4 and H1 strains were detected in Japan. A G3 strain was also detected in 2011. Three outbreaks have occurred in Japan in 2012: D8 outbreak in Aichi-Gifu, Chiba, Miyazaki; D9 outbreak in Okayama; and H1 outbreak in Fukushima. Most cases were considered to be imported or import-related cases, although epidemiological information was not available for all cases, reiterating that epidemiological investigation should be strengthened.

(2) Virological surveillance of measles in China

Dr Zhang Yan from China CDC presented updates on measles surveillance in China. From 1993 to 2012, 2800 measles strains were genotyped and seven genotypes were found: H1, H2, A (vaccine), D4, D8, D9, D11 and H1 strains. The H1 strains detected predominantly from 1993 have been circulating for at least 20 years in China. Measles genotype information is available from 30 provinces except Tibet. Since 2009, multiple imported measles genotypes (D4, D8, D9,
D11) have been found in five provinces. Dr Zhang presented information on a measles outbreak caused by new D11 measles strains in Menglian, a county of Yunnan Province, which has a border with Myanmar, but in 2012, D11 measles strain was not found in Yunnan. It was presumed that increased immunity of local population prevented the spread of imported viruses.

Several SIAs were conducted throughout China, especially in 2010, when nationwide synchronized SIAs were conducted and 100 million children were immunized. In 2011, SIAs were conducted in more than 400 counties with relatively high intensity. The laboratory performed an analysis on the endemic H1 strain to know whether the circulating pattern of endemic H1 viruses had changed following these SIAs. The results showed that the circulating pattern changed after the SIAs in 2010 and 2011, and by 2012, the diversity of circulating strains had been reduced and more similar viruses were circulating. Though few variants were detected, the circulation was in very limited areas and caused limited spread in 2012. It was concluded that SIAs are efficient measure to stop the spread of viruses rapidly.

(3) Australia experience

Mr Matthew Kaye from VIDRL presented molecular surveillance of measles viruses in Australia from January 2011 to December 2012. Upon request from public health facilities, VIDRL performs free PCR on samples with an IgM positive result, including genotype confirmation for interstate laboratories that 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. In 2011, 506 samples were tested by real-time PCR. Of those tested, 150 (29%) samples were positive and 105 of them were genotyped. The measles genotypes detected were: A (vaccine, n=7), D4 (n=31), D8 (n=26), D9 (n=40) and H1 (n=1). In 2012, 410 samples were tested by real-time PCR and 149 (36%) samples were positive. Eighty-five of them were genotyped and six measles genotypes were detected: A (n=2), B3 (n=5), D4 (n=1), D8 (n=70), D9 (n=6) and G3 (n=1). A measles D8 outbreak in New South Wales was the largest in 14 years following importation from Thailand. Most cases were among Australian born under-immunized children.

Mr Kaye also stated that vaccine was widely available in Australia in the early 1970s, yet age distribution of measles cases shows that people over 40 years old who missed vaccination before are also infected. Some clustering of cases under 44 years old suggests that the measles vaccination programme is still missing some people and herd immunity may not be high enough to prevent transmission.

(4) Republic of Korea experience

Dr Kisoon Kim from Korea CDC presented the Republic of Korea’s strategy of molecular epidemiology for the measles virus, and talked about a molecular epidemiological analysis of a 2007 nosocomial outbreak, modified measles cases in 2010, and identification of imported cases. Real-time PCR is used as a supplementary diagnostic tool, and conventional RT-PCR and sequencing are done for genotyping. The measles outbreak in 2007 involved 180 confirmed cases out of 451 suspected cases. About 45% of cases were infected via a nosocomial transmission and involved 81 cases from six hospitals; 76% of cases were under two years and 69% of cases were unvaccinated. Several lessons were learnt from the 2007 outbreak, including: nosocomial transmission can play an important role in countries that have eliminated or are close to eliminating measles; timely vaccination is required to prevent such outbreak; and a high-quality
laboratory surveillance system with molecular approaches is critical to detect and interpret measles prevalence. D5 and H1 strains were detected during the 2007 outbreak. In 2010, an outbreak of measles in a middle school was confirmed by measles IgM or RT-PCR. Most of 78 cases did not have typical clinical signs of measles and paediatricians did not have an initial diagnosis of measles. Measles IgG testing was performed on paired sera from 14 cases with positive RT-PCR and negative measles IgM results. Increased IgG titer were detected from all 14 cases. Measles IgG avidity testing would have been useful to interpret these cases. Also, rapid response through molecular diagnosis is useful during the early phase of an outbreak due to inadequate serological information.

Dr Kim also shared the results of molecular characterization of measles viruses from 2007 to 2011. The results showed that the H1 strains detected were related to Chinese strains and the D5 strains were related to Japanese strains. In 2008–2009, when the measles virus was inactive in the Republic of Korea, two measles strains were detected. The B3 strain was detected from a Libyan national, and the H1 strain was detected from a person who came from Viet Nam. In 2011, 16 clustering cases of measles were reported in Changwon city, Gwangnam, where many foreign workers live. D9 strains were detected. This was the first detection of D9 strains in the Republic of Korea since genotyping was conducted in the country. It was highlighted that an additional strategy for genetic analysis should be developed to visualize imported cases within genotype.

(5) Molecular surveillance of measles in the United States of America

Dr Paul Rota from US CDC presented on enhancing molecular surveillance for measles in the United States of America. Molecular epidemiologic studies are a key component of verification of measles elimination, and the target is to obtain genotype information from at least 80% of chains of transmission. In 2012, genotype information was obtained from four out of four outbreaks (100%); however, genotype information was obtained from only 12 (42%) out 28 non-outbreak cases. Collecting samples from sporadic cases can be difficult. During 2011, the United States of America reported the highest number of measles cases in 15 years. There were 222 measles cases reported from 31 states associated with importations, and 200 (90%) cases were laboratory-confirmed. Only 37 (19%) cases were tested for detection of measles virus RNA. In 2012, 55 measles cases were reported (provisional data) and 48 (87%) cases were laboratory-confirmed. Measles virus RNA was detected from only four (8%) cases. Dr Rota also presented the results of the measles real-time RT-PCR done on samples collected every day within a month during 2011 and 2012, and the results showed that measles RNA can still be detected up to 28 days; however, a high positivity rate is found in samples collected within nine days from onset of illness.

Measles genotypes in the USA from 2011 to 2012 were reported. Seven measles genotypes were detected in the Region of the Americas in 2011: A(vaccine), B3, D4, D8, D9, G3 and H1. G3 and H1 strains were not detected in 2012. B3 strains were imported from Iran (Sistan), Germany and Africa (Cameroon); D4 from the United Kingdom of Great Britain and Northern Ireland (Manchester); and D8 from Europe, India (Viluppuram), Thailand and the United Kingdom of Great Britain and Northern Ireland (Belfast) in 2012.

Dr Rota described the real-time RT-PCR kit for the detection of measles RNA. It was noted that real-time RT-PCR is more sensitive than conventional (endpoint) RT-PCR, but sequence information from the conventional PCR product is required for genotype assignment and confirmation of vaccine reactions. The real-time PCR product is not suitable for sequence analysis. A new version 2.0 of the measles genotyping kit, which contains primers that can be used for sequencing, was also developed.
2.2.4 Country and area reports

(1) Cambodia

Dr Buth Sokhal, Deputy Director, National Institute of Public Health (NIPH), presented on measles and rubella surveillance in Cambodia. The national measles/rubella laboratory receives samples from all provincial/referral hospitals and health centre sites. Dried blood spot (DBS) samples are collected from children younger than one year of age and serum samples from children older than one year of age. Serological test using Siemens measles and rubella IgM ELISA kits on serum and DBS samples were conducted. During 2010, an increase in measles cases were noted, and among 2580 samples tested for measles and rubella IgM, 416 (16%) were positive for measles and 72 (2.8%) were positive for rubella. There was also an increase in rubella activity in 2011, and a total of 3202 samples were tested. Of these, 1048 (32.73%) were positive for rubella and only 288 (8.99%) were positive for measles. Throughout 2012, no measles positive case was confirmed; however, 184 (18.70%) were positive for rubella. The highest percentage of rubella cases (31.1%) was detected in people aged 15 years and older, while the highest number of measles cases was identified in children aged five to 14 years old.

The laboratory has implemented the use of in-house control, confirmatory testing by Hong Kong (China) RRL and proficiency testing as quality assurance measures, as recommended by WHO. The PT score was 100% for both measles and rubella from 2010 to 2012. Confirmatory testing scores were 100% for measles and >90% for rubella from 2010 to 2012. Measles immunization coverage from 2008 to 2012 has improved from 89% to 94%.

(2) Fiji

Dr Prem Singh, acting Senior Medical Officer, presented on immunization and surveillance in Fiji. A monovalent measles vaccine was introduced in 1982 for children aged nine months. After an outbreak of measles in 2002, measles and rubella (MR) vaccine was given to children aged 12 months and at school entry (4 or 5 years old) from 2003. A rubella outbreak occurred from June to December 2011, and out of 529 suspected cases, 153 (28.92%) were confirmed cases. Health workers were given MR vaccinations (1% of population). Mandatory reporting of measles started in 1976, and in 2007, laboratory-based surveillance was initiated.

Mrs Talica Vakacolata, Senior Laboratory Technician from Mataika House, Fiji presented on the laboratory activities of the national measles/rubella laboratory. The laboratory in Mataika House receives samples from three subdivisional hospitals/health centres and pathology laboratories in the Colonial War Memorial Hospital, Lautoka Hospital and Labasa Hospital. Mataika House tested 541 samples in 2011, 180 samples in 2012, and 13 samples as of March 2013. Only two samples were measles IgM positive in 2011; no measles case has been detected since 2012. From rubella outbreaks in 2011, 544 samples were tested and 153 (28.12%) were rubella IgM positive. In 2012, 183 samples were tested and 25 (13.66%) were rubella IgM positive. CRS surveillance is also being conducted through the Paediatric Department on a case-by-case basis, informal system (not captured in notifiables), and post-rubella outbreaks data (1995, 2002, 2006). Siemens IgM ELISA kit is used for the serological diagnosis of measles and rubella. The results of confirmatory testing from VIDRL showed 83% concordance. The results of proficiency testing were 100% for both measles and rubella. 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) Lao People's Democratic Republic

Dr Phengta Vongphrachanh from the National Center for Laboratory and Epidemiology (NCLE) presented the measles and rubella surveillance activities in the Lao People’s Democratic Republic. Single-dose measles vaccination at 9–11 months is administered through routine childhood immunization, and additional SIAs are regularly conducted. The routine vaccine coverage rate was 68% in 2012. In December 2012, mop-up activities in 25 high-risk districts were conducted. Acute fever and rash is one of 17 notifiable diseases in the Lao People’s Democratic Republic. Any case of acute fever and rash must be reported by provincial and district surveillance, and NCLE provides the results within seven days. NCLE does not have capacity for molecular testing/genotyping and virus isolation for measles and rubella; however, equipment for molecular work such as conventional PCR, real-time PCR and luminex PCR are available and could be used if required.

The Siemens IgM ELISA kit is used for detecting measles and rubella IgM antibodies. Since 2007, measles IgM negative samples are also being tested for rubella IgM. In 2010, samples from 85 cases, which accounted for only 26% of suspected cases, were tested in NCLE. During 2011, 183 samples were tested. Of these, 10 (5.46%) and 9 (4.92%) were positive for measles and rubella, respectively. In 2012, there was an increase in the number of samples, and out of 402 samples tested, 10 (2.49%) were positive for measles and 50 (12.44%) were positive for rubella. The laboratory scored 95% for measles and 100% for rubella in the 2012 WHO PT. A proportion of tested samples were sent to Hong Kong (China) RRL for confirmatory testing in 2012, and the concordance rates were 98% for measles and 82% for rubella. The use of in-house control samples for ELISA has not been implemented yet. The timeliness and completeness of surveillance and laboratory reporting have been improved to 91% and 100%, respectively, in 2012. Main challenges identified are: (1) improving data management and coordination with other units; (2) providing refresher training for provincial and district staff on field investigation and collection of blood specimens; (3) using in-house control samples for measles and rubella IgM testing for quality assurance; (4) strengthening laboratory biosafety; (5) ensuring timely procurement of kits; and (6) receiving WHO accreditation (the laboratory is implementing recommendations from the last on-site review to be fully accredited by WHO).

(4) Malaysia

Madam Janagi Naidu from the National Public Health Laboratory (NPHL) presented the measles elimination programme in Malaysia. Measles elimination started in 2004 with specific objectives to maintain the number of susceptible individuals below the critical number required to sustain transmission of the virus, eliminate measles by year 2015 and achieve zero measles mortality. There was no case of measles mortality in 2010, but there was an isolated incidence of four deaths in Sabah involving foreigners in 2011. No measles death was reported in 2012. The measles, mumps and rubella (MMR) vaccine was introduced in 2002. Two doses of MMR vaccine are given to children at 12 months and seven years of age in Peninsular Malaysia and Sarawak. In Sabah, one dose of measles vaccine is given at six months followed by two doses of MMR vaccine at 12 months and seven years of age. Measles incidence has decreased since the introduction of MMR; however, in 2011 and 2012, there was a notable increase in measles cases due to outbreaks. Measles cases by month of rash onset from 2012 to February 2013 showed 1655 (89%) measles cases were laboratory-confirmed, 156 (8%) were clinically confirmed and 57 (3%) were epi-linked. Most of the cases from Selangor were aged six months to seven years old, with the most affected age group being one-year-old children. The number of cases has increased due to poor herd immunity and good reporting.
Samples for confirmatory testing were sent to Hong Kong (China) RRL, and concordance rates were 100% for measles and >95% for rubella in 2011 and 2012. Results of the 2012 PT were 100% for measles and 85% for rubella. NPHL is using Siemens kits for measles and rubella IgM and IgG testing. Virus isolation is also performed using Vero/hSLAM cells and confirmed by direct immunofluorescence assay. Molecular detection using RT-PCR for measles and rubella is also implemented. Laboratory data showed that in 2011 and 2012, 4227 and 4486 samples were tested for measles, respectively. Of these, 1182 (28%) were measles IgM positive in 2011 and 1320 (29%) were measles IgM positive in 2012. There was a decrease in rubella cases from 42% in 2009 to 13% in 2012. Samples for virus isolation have also increased from 124 in 2009 to 1576 in 2013. Positivity for virus isolation was 6% in 2011 and again in 2012. Additional genotyping of samples from this laboratory was conducted by Hong Kong (China) RRL. Measles genotypes G3, B3, D9 and A(vaccine-related) and rubella genotype 2B strains were detected in 2010; however, in 2011, B3 disappeared and D8 was detected instead. In 2012, only D8 and D9 measles strains were detected, while 2B and 1E rubella strains were identified. Malaysia has appointed a measles subnational laboratory in Sabah to conduct serology testing for measles and rubella IgM for East Malaysia, and NPHL is monitoring the quality of testing in this laboratory to ensure it adheres to the requirements of WHO.

(5) Mongolia

Dr Rentsen Tuul from the National Measles Laboratory of the National Center for Communicable Diseases (NCCD) presented on measles/rubella laboratory surveillance and immunization programme in Mongolia. MCV1 was introduced in 1973, and MCV2 was introduced in 1986. In 1996, active surveillance for measles was introduced with integration of AFP surveillance. In 1998, a mandatory reporting system of measles started. Genotype data have been available since 2001. The last outbreak of measles occurred in Mongolia during 2001–2002, and in 2007 and 2012, small outbreaks of rubella were reported. MMR vaccination was introduced in 2009 and is provided for children aged nine months and two years old. After the introduction of MMR, the last measles case was detected in 2009. In 2012, vaccine coverage reached 98.3% after the national immunization days were conducted. Rubella vaccine was introduced in 2001 in Ulaanbaatar City for children aged three to 17 years old and women aged 18–34 years, and vaccination rates reached 96.6% and 86.7%, respectively.

The laboratory has been accredited by WHO since 2004 as the national measles laboratory. It has the capacity to perform serology, virus culture using Vero/hSLAM cells and molecular detection by PCR methods. For serology, Siemens IgM ELISA and indirect immunofluorescence assay are used. During 2012, out of 627 samples tested, 240 (38.28%) were positive for rubella. During 2013, out of 20 samples tested, no rubella IgM was detected as of 5 March. The laboratory conducted mumps diagnosis from 2009 to 5 March 2013, and the mumps IgM positive rate was very high at 48.31%. Parvovirus B19 diagnosis is also being performed. During 2011–2012, 50 samples were tested for parvovirus and six (12%) were IgM positive. To obtain baseline measles genotype information in Mongolia, measles IgM positive serum samples and a few isolates were sent to Hong Kong (China) RRL for genotyping. Genotype H1 strains were detected in 2009. Rubella genotype 1E strains were detected from 2010. Mumps F and H3 genotype strains were detected in 2011; however, only a genotype F strain was detected in 2012.

The laboratory participated in various WHO quality assurance programmes and scored 100% in recent confirmatory and proficiency testing. NCCD also performed the measles and rubella FTA practice panel using RT-PCR and real-time RT PCR. The score for measles
RT-PCR was 87.5% (7 out of 8) and the score for or evaluation of real-time results was 81.82% (9 out of 11). The score for rubella RT-PCR was 100% (7 out of 7) and score for or evaluation of real-time results was 90% (9 out of 10). Two training workshops were conducted in 2011 and 2012 to enhance laboratory capacity in the diagnosis of measles and rubella.

(6) New Zealand

Ms Sheryl Young from Canterbury Health Laboratories presented on the immunization programme and laboratory activities in New Zealand. Immunization coverage is measured at ‘milestone ages’ using National Immunisation Register (NIR) data. The milestone ages are six months, eight months, 12 months (one year), 18 months, 24 months (two years) and five years of age. As of December 2012, immunization coverage data showed that 91% of children aged 12 months (one year) and 24 months (two years) received all of their age-appropriate immunizations. Two doses of MMR vaccine are recommended: one at 15 months and another at four years of age. During 2009, measles outbreaks occurred in Dunedin (H1 strain) and in Christchurch (D4 strain). In 2011, three importations of measles virus (D9, D8 and D4 strains) occurred from January to June. D9 importations into Auckland, Wellington and Christchurch were followed by D9 importation into Hawkes Bay and a D4 importation into the Auckland area. D4 importations in Auckland, Wellington (mini) and Hawkes Bay continued until July 2012.

Diagnostic methods used are: measles real-time RT-PCR and virus isolation in Vero/hSLAM cells and RT-PCR for rubella. Types of samples tested include: throat swab/nasopharyngeal swab, urine, white blood cells, serum and oral swabs. Parvovirus B19 detection is included in the testing strategy as differential diagnosis of measles IgM positive samples from the referring laboratory. Siemens ELISA kit is used for detection of measles and rubella IgM. For measles and rubella IgG tests, Biomerieux MiniVidas kits are used. Other laboratories in New Zealand are using Microimmune IgM, Siemens IgM and TRINITY Captia IgM kits for measles IgM diagnosis, while Abbott Architect IgM, Siemens IgM and Vidas Rubella IgM kits are used for rubella diagnosis.

The total number of samples tested for measles is unknown since reporting and sending samples to the national measles laboratory is voluntary. Data are derived from actual tests at Canterbury Health Laboratories and cases reported from other laboratories. Genotyping is performed where possible. In 2012, nine samples were genotyped for measles: seven samples with D4 strain from the 2011 outbreak, one with D8 strain from traveller returning from Thailand, and one with A strain from post vaccination. There were six rubella-positive cases in 2012 and no CRS case was found. Canterbury Health Laboratories implemented in-house controls for serological testing and participated in various external quality assurance programmes. In 2012, the laboratory scored 100% on both proficiency and confirmatory testing.

(7) Papua New Guinea

Ms Janlyn Kumbu from the Central Public Health Laboratory (CPHL) presented an overview of the measles and rubella surveillance activities in Papua New Guinea. No documented measles outbreak has been reported since 2005; however, the reported vaccination coverage of children aged nine months was around 65% in 2011. IgM ELISA testing is performed using Siemens kits supplied by WHO. Measles and rubella testing is performed in parallel. There has been no laboratory-confirmed measles case since 2008, but an increasing number of rubella-positive cases have been detected. During 2011–2012, CPHL tested 84 samples and 19 (22.62%) were positive for rubella. The laboratory participated in WHO’s proficiency and confirmatory testing programme for quality assurance and was recently accredited by WHO. In 2011 and 2012, the laboratory scored 100% for both measles and rubella
PT. Biosafety training for national and provincial laboratories will be conducted in 2013. The laboratory will strengthen its PCR capacity in VPD surveillance as the facility is available.

(8) Philippines

Mr Rex Centeno from RITM presented on the immunization and surveillance activities of the Philippines. For routine immunization, the monovalent MCV is given to children at nine months as the first dose (MCV1) and MMR at 12–15 months old as the second dose. The Philippines experienced large outbreaks in 2010 and 2011. Among 2881 laboratory-confirmed cases in 2010 and 3238 laboratory-confirmed cases in 2011, only D9 measles virus strains were detected. The majority of measles cases in 2011 were from the National Capital Region (NCR) as well as regions 1, 3, 4A, 5 and 11. The majority of measles cases in 2011 were among children under two years old. A measles/rubella SIA was conducted from 4 April to 31 May 2011. This door-to-door campaign covered children aged nine to 95 months. Surveillance data during the latter half of 2011 and early 2012 showed a few provinces with residual measles virus transmission. A total of 1499 cases were reported in 2012. Of these, 594 were laboratory-confirmed, five were epi-linked and 900 were clinically confirmed. Most of measles laboratory-confirmed cases in 2012 were from Region 6. Rubella cases also decreased from 1115 laboratory-confirmed cases in 2011 to 150 laboratory-confirmed cases in 2012. Rubella confirmed cases in 2012 were from NCR and regions 4A, 6, 7 and 11.

The laboratory is capable of performing serology on serum samples and DBS and cell culture from throat/nasopharyngeal swabs. Samples for virus isolation were also received from the sentinel sites; however, regions with high incidence of measles failed to collect samples for virus isolation. Hong Kong (China) RRL conducts the molecular detection for measles and rubella genotyping for the Philippines. Additional genotyping results were obtained from Hong Kong (China) RRL using IgM positive or equivocal serum samples. In November 2012, the laboratory received measles and rubella FTA practice panels. The results of the measles FTA practice panel were 100% for RT-PCR and genotype assignments; however, one point was subtracted for the evaluation of real-time PCR results (10 out of 11 scores) because sample 4 (blank FTA disk with no lysate) was positive, indicating cross-contamination during RNA extraction or PCR set-up. Rubella FTA practice panel results were 100%. 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 and internal control samples was monitored. Timeliness in reporting within seven days was 88.5% in 2012.

(9) Republic of Korea

Dr Kim Kisoon from Korea CDC presented the vaccination and surveillance activities in the Republic of Korea. To eliminate measles, trivalent MMR vaccine has been used in the Republic of Korea. The recommended age for routine vaccination is 12–15 months for the first dose of MMR, and four to six years for the second dose of MMR. The vaccination rate for two doses of MMR has reached ≥95%. The laboratory performs serology, virus isolation and molecular detection using real-time PCR and sequencing for confirming measles and rubella infection. Differential diagnoses for parvovirus B19 and human herpesvirus 6 (HHV-6) are also performed using Biotrin IgM EIA (parvovirus B19) and Panbio IgM EIA (HHV-6). From the outbreak in 2011 in Gyeongnam, all RT-PCR measles positive cases were identified as D9 measles virus strain except one vaccine-related case. This was the first detection of D9 strains in the Republic of Korea, which was possibly related to importation. In 2012, no measles virus was detected from measles IgM positive sera tested by RT-PCR, since it is difficult to collect good-quality serum samples and other samples such as throat swabs and urine.
The laboratory participated in the WHO proficiency and confirmatory testing programme for quality assurance. The accuracy of the recent confirmatory testing for measles and rubella IgM and molecular PT was 100%. 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 and internal positive control samples was monitored. This laboratory is also responsible for external quality assurance of provincial health and environmental research institutes (PHERIs). It sends PT panel samples for serology, virus isolation and RT-PCR to PHERIs, and PT panel samples for serology to five major private diagnostic centres. The panel samples for serology are validated and reconfirmed by VIDRL before their distribution. The panel samples for virus isolation and RT-PCR are composed of three positive samples, namely, human rhinovirus (type A), measles virus (genotype H1) and rubella virus (Wistar RA 27/3), and three negative samples (minimum essential medium only). In 2012, panel samples were tested in 10 out of 17 PHERIs using Siemens ELISA IgM kits, Vero/hSLAM and RT-PCR, and in five private diagnostic centres using Radim (two centres), Euroimmun (two centres) and Biorad (one centre) IgM ELISA kits. Ten PHERIs and four private diagnostic centres reported final results within 35 days after distribution, but one private diagnostic centre missed the deadline. All of the participating PHERIs reported good results. The results of most private diagnostic centres showed 100% sensitivity, but only 67% specificity.

(10) Singapore

Dr Lui Sook Yin, Scientific Officer from Singapore General Hospital, presented on the vaccination and surveillance programme in Singapore. Measles and rubella vaccination was introduced into the national childhood immunization programme in 1976. At that time, it was recommended to give one dose of measles vaccine to children aged one to two years old, and one dose of rubella vaccine to pre-adolescent females aged 11–12 years old. In 1985, a law was passed making measles immunization compulsory for children aged one and two years old. Since then, the number of measles cases has dropped. In January 1990, monovalent measles was replaced by MMR. In 1992, a resurgence of measles cases was noted, with a shift in peak age distribution to older children, youths and young adults; as a response, a catch-up immunization initiative was implemented for students aged 12–18 years old. In January 1998, a two-dose MMR vaccination regime was implemented, with the first dose at one to two years old and the second dose at 11–12 years old. In 2008, the timing of the second dose was brought forward to six to seven years old. Effective 1 December 2011, on recommendation by the Expert Committee on Immunization, children receive MMR at a younger age; first dose at 12 months of age and second dose at 15–18 months of age. National vaccination coverage for MMR is maintained at around 95% for the first dose, and above 90% for the second dose.

The Virology Laboratory in the Pathology Department of Singapore General Hospital serves as the national measles laboratory 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. An enhanced surveillance programme has been set up by the Ministry of Health to improve the measles surveillance system in Singapore. Samples negative for measles IgM are tested for rubella IgM, and samples negative for rubella IgM are tested for measles IgM. An increase in measles cases was noted in 2011, when 55 (23.1%) out of 238 samples tested were positive for measles IgM. In 2012, 249 samples were tested for measles IgM. Of these, only 14 (5.6%) were positive for measles. No measles case has been detected in 2013 as of January. Measles genotyping revealed H1, G3 and D9 strains in 2010; G3, D4, D8 and D9 strains in 2011; and D9 strains in 2012. The data on rubella showed low cases of rubella from 2011 to January 2013. Out of 810 samples tested for rubella IgM, 33 (4.1%) were positive for rubella.
The Singapore General Hospital 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 evaluated and monitored for each run, and results are assessed and validated before reporting. Monitoring and maintenance of equipment are regularly performed. Confirmatory testing by Hong Kong (China) RRL showed 100% concordance in 2011 and 2012, and WHO PT results were 100% from 2010–2012. The laboratory scored 100% on the 2012 measles and rubella molecular FTA practice PT. The laboratory is also accredited by the College of American Pathologists, Joint Commissions International and WHO.

(11) Viet Nam

The National Institute of Hygiene and Epidemiology (NIHE) in Ha Noi and Pasteur Institute in Ho Chi Minh City serve as the national measles and rubella laboratory for northern and southern Viet Nam, respectively.

NIHE: Dr Dinh Tuan Duc from NIHE presented the vaccination programme in Viet Nam and the surveillance system in Ha Noi. Since 2011, two-dose measles vaccination has been part of routine immunization in Viet Nam; the first dose is given to children at nine months, and the second dose is given to children at 18 months. From 2013 to 2015, rubella vaccination will be introduced into the National Programme on Immunization to children from nine months to 14 years old. The rubella vaccine is supported by the Global Alliance for Vaccines and Immunization. Two measles IgM positive cases were reported in 2012; however, during investigation, it was concluded that the measles cases were vaccine-related IgM response. In 2011, an increase in rubella cases was detected. Among 2236 samples tested for rubella IgM, 1399 (57%) were positive for rubella. In 2012, among 406 samples tested for rubella IgM, 89 (22%) were positive for rubella. A small rubella outbreak also occurred in Lai Chau province during 2012, and patients were children aged 13–14 years old in the same school.

Quality assurance measures including the use of in-house control samples, confirmatory testing by Hong Kong (China) RRL and participation in WHO PT were well implemented. The laboratory has obtained a 100% PT score since 2006. Confirmatory testing results for measles and rubella also showed >90% concordance in 2010 and 2012; in 2011, the rubella concordance rate was 84%. Virus isolation and molecular detection of measles and rubella by RT-PCR were also performed. In 2010, among 28 samples tested, virus isolation showed three (11%) samples had cytopathic effects, and 25 (89.28%) samples were positive for measles by RT-PCR. Among 55 samples tested by virus isolation and RT-PCR, no measles virus was detected in 2011, but 31 (56%) were positive for rubella by RT-PCR. In 2010, 21 H1 measles genotype strains and 16 2B rubella strains were detected in 2011 in northern Viet Nam. CRS surveillance was initiated in northern Viet Nam with one sentinel hospital (National Children’s Hospital) and in southern Viet Nam with two sentinel hospitals (Children's Hospital No.1 and Children’s Hospital No.2) in 2011. A total of 199 CRS samples were tested for rubella IgM, and of these, 168 (84.42%) were positive for rubella.

Pasteur Institute, Ho Chi Minh City: Ms Pham Thi Nhung from Pasteur Institute, Ho Chi Minh City presented on measles and rubella surveillance in southern Viet Nam. After a large outbreak in 2009, the number of measles cases dropped in 2010–2012: 885 (20.82%) laboratory-confirmed cases among 4250 samples tested in 2010; six (<1%) cases among 1467 samples tested in 2011; and one (<1%) case among 352 samples tested in 2012. However, there was a notable increase in rubella activity: 1880 (47.8%) cases in 2010 and 1059 (62.9%) cases in
Only 25 (7.1%) out of 352 samples were positive for rubella in 2012. No measles or rubella case was detected from January to February 2013. Timeliness of testing within seven days improved from 24% in 2011 to 97% in 2012. Surveillance for congenital rubella syndromes in southern Viet Nam began in 2011. The data showed that in 2011, 82% of suspected CRS cases were positive for rubella IgM and 56.5% of CRS cases had IgG antibodies, while in 2012, 43% were positive for rubella IgM and 53.8% had IgG antibodies. However, the presence of IgG antibodies is considered insignificant, as a second sample was not collected for demonstration of rise in antibody titre. Virus isolation and RT-PCR for measles and rubella were performed on a few samples in 2011 and 2012, and the results were all negative. Among 99 samples of clinically suspected CRS cases, two samples were positive for rubella by RT-PCR and one rubella isolate was confirmed and sequenced. The H1 measles strain and 2B rubella strain were detected in southern Viet Nam. In-house control samples were used for measles and rubella IgM assays. Proficiency and confirmatory testing scores for measles and rubella in 2011 and 2012 were 100%. An ISO 15189 certificate of accreditation was acquired in 2011. Results of the molecular FTA practice panel PT on measles and rubella were excellent, except for the score/evaluation of real-time PCR for measles, which was 10/11 (90.91%).

**Pasteur Institute, Nha Trang:** Mrs Huynh Kim Mai from Pasteur Institute, Nha Trang presented on the laboratory diagnosis of measles and rubella virus infection in central Viet Nam. Pasteur Institute in Nha Trang serves as a subnational measles laboratory. The laboratory has the capacity to perform serology, virus culture using Vero/hSLAM cells, RT-PCR methods and genotyping. For serology, Siemens IgM ELISA kits are used. In-house control was prepared and well implemented in the serological testing. The subnational measles laboratory participated in WHO PT as part of its quality assurance programme and scored 100% in 2012. During 2012, a decrease in measles and rubella cases was noted. Only 130 samples were tested for measles and rubella IgM. Of these, three (2.3%) samples were positive for measles IgM and seven (5.38%) samples were positive for rubella IgM. There was a measles outbreak in 2010, when 279 (40.91%) out of 682 samples tested were positive for measles IgM. Similarly, a rubella outbreak occurred in 2011, when 338 (34.95%) out of 967 samples tested were positive for rubella IgM. All positive, equivocal samples and 10% of negative samples are sent to NIHE for confirmation testing. Measles and rubella genotyping on isolates in 2010 detected HI measles strain and 2B rubella strains.

### 2.2.5 Use of alternative sampling: DBS, oral fluid and point-of-care test

Dr Dhan Samuel from the Virus Reference Department, HPA, United Kingdom of Great Britain and Northern Ireland, presented alternative sampling and point-of-care tests (PoCT) in the diagnosis and surveillance of measles and mumps. He emphasized the two aspects of surveillance, namely: (1) serological assays (IgM tests for confirmation of acute infection and IgG tests for seroprevalence) using serum samples as gold standard; and (2) molecular epidemiology (PCR assays) for confirmation of acute infection, tracking of epidemiological pathways, surveillance of virus diversity and investigation of potential adverse events using various samples such as blood, urine, nasopharyngeal aspirate, throat swabs, buccal swabs, and cerebrospinal fluid. Alternative sampling is important because collecting clinical samples from infants, particularly venous blood, is very challenging. Lack of cold storage and proper shipment of samples are also considerations for using alternative sampling. DBS and oral fluids are two alternatives to serum sampling. Collection of oral fluids with Oracol™, a device produced by Malvern Medical Developments, and processing of oral fluid samples for serology and RT-PCR were discussed. Oral fluid samples were first used in 1992 for the detection of measles, mumps,
and rubella antibodies using antibody capture radioimmunoassay.\textsuperscript{2} Though the immunoglobulin concentration in oral fluids is low (compared to immunoglobulin level in serum), sensitive EIAs to detect IgG and IgM were developed using recombinant capsid proteins derived from baculovirus. Using microimmunecapture IgG and IgM assays (GAC-ELISA), oral fluid samples were evaluated for measles antibodies. For measles IgG, oral fluid samples showed 90.3% sensitivity and 97.4% specificity, while for measles IgM, oral fluid samples showed 100% for both sensitivity and specificity.\textsuperscript{3} As oral fluid is a heterogenous sample, a total IgG is measured to determine sample quality. Oral fluid samples are not only used for serological assays, but also can detect measles RNA for up to 28 days post onset by PCR.

Dr Samuel also presented on the development of mumps IgM PoCT based on lateral-flow immunochromatography. A mumps epidemic in 2005 in the United Kingdom resulted in a large number of oral fluid samples being sent to the reference laboratory. These samples enabled the development of a PoCT based on oral fluid testing. The mumps IgM PoCT evaluated 196 oral fluid samples received for UK MMR surveillance program, and the results revealed 79.5% sensitivity and 100% specificity.\textsuperscript{4} A measles PoCT was also developed with the same format and principle as the mumps IgM PoCT. An evaluation of the measles PoCT was done on 170 serum samples, and the results showed 90.8% sensitivity and 93.6% specificity. Also, an evaluation of the measles PoCT for oral fluid samples was performed. A total of 282 oral fluid specimens were received in the United Kingdom during 2008 as part of surveillance programme. A comparison of measles IgM PoCT and capture EIA results on oral fluids showed 88.6% sensitivity and 90.4% specificity. PoCTs have potential to make a significant contribution to measles surveillance. Based on comprehensive validation, oral fluid samples provide a unique opportunity to enhance molecular surveillance as well as provide case confirmation for suspected measles cases. For serological assay of oral fluid samples, Microimmune IgM ELISA kit should be used. Quality assurance programmes for DBS and oral fluids need to be established.

2.2.6 MeaNS database

Dr Miguel Norman Mulders, scientist from WHO Headquarters, presented the details of the MeaNS database. WHO genotype data will be phased out to eliminate the need for multiple reporting. The MeaNS sequence database was established in 2008 and is managed by HPA in consultation with WHO for storing measles sequences, and comparison/phylogeny of viruses circulating in countries that report to WHO. The MeaNS database has the built-in ability to upload data to GenBank and has restricted access (only members of WHO measles and rubella laboratory network can submit and view sequence data according to the terms and conditions). Data on 9798 measles sequences/genotypes have already been deposited into MeaNS, and of these sequences, there were 9632 measles sequences of the 450 nucleotide (nt) region of the viral nucleoprotein (N) gene and 592 measles sequences covering the full H gene. All six WHO Regions are submitting sequences, but a majority of the sequences in 2010 came from the European Region (n=around 3000), followed by the Western Pacific Region (n=around 1300).


Users of the MeaNS database can access measles news and articles, some of which can be obtained without registering. Registration to MeaNS is free for users from academic and non-profit institutions, based on the terms and conditions given in the academic license. Commercial users are advised to register their interest and are given access to the restricted areas of the database on a case-by-case basis, depending on their access needs. Steps involved in the submission of sequence data were discussed. Sequence files are currently stored as American Standard Code for Information Interchange (ASCII) data that can be submitted to GenBank on the user’s discretion. Sequences are stored according to the WHO naming convention, as stipulated in the Weekly Epidemiological Record in March 2012. Two different sequences cannot have the same WHO name. The MeaNS database will generate the name if relevant information is provided, but once submitted, the WHO name cannot be corrected by the user. If a correction to the name is needed, a request has to be submitted by e-mail to the MeaNS Administrator (Means-Admin@hpa.org.uk).

A new tool added to the MeaNS database allows users to submit data directly to GenBank. Users can check the data automatically generated by MeaNS before clicking on the submit button to format the data for GenBank (upon users’ discretion). GenBank is a sequence database that is open to the public. The Basic Local Alignment Search Tool (BLAST) can be used to identify viruses that are closely related or identical. There are some issues that are being fixed. Users can download results in spreadsheet format, but not for the actual sequence. Future plans include the development of a user manual and a Rubella Nucleotide Surveillance (RubeNS) database. Dr Mulders also demonstrated the use of the data to understand how well identical viruses spread at the global level and exactly how big the problems are.

2.2.7 Measles/rubella serosurvey

(1) Standardization of measles serosurvey

Dr Miguel Norman Mulders presented the methodology of monitoring immunization programmes through serosurveillance. He emphasized the significance of sero surveillance as this will help validate vaccine coverage estimates, generate population susceptibility profiles and identify at-risk populations and immunity gaps. Therefore, it is an important tool for programmatic functions and political/administrative functions. A reference material from WHO, entitled: Documenting the impact of hepatitis B immunization: best practices for conducting a serosurvey, was discussed. The success of the serosurvey will depend on the sampling method used such as target population, samples, laboratory test and result analysis. Target population considerations include: (a) information on exposure/immunization probability and known waning immunity of the whole population, (b) geographically/culturally defined group (compliance and political aspects), (c) specific age group/at-risk population, (4) historical/current (predictive and real-time monitoring). Sample scheme should also be considered based on the targeted collection or convenience samples, type and volume, immediate sample treatment, sample storage and transport, and sample data collection and recording. The tests used for detecting the IgG disease-specific antibodies should have high sensitivity and specificity. Other considerations for choice of test include sample volume limitations of some tests (for example, EIA, radioactive immunoassay); expertise and equipment availability; and if tests are done either manually or automatically, all of which have an effect on timeliness of availability of results. Considerations on the results analysis of the serosurvey include sample size (statistical confidence/resource

availability), selection criteria, avoiding selection bias, statistical package and confidence of interpretation. It was concluded that serosurveillance offers potential for monitoring and driving immunization programmes. Planning an effective serosurvey is complex process, but it’s possible with a systematic approach. From available published data, it appears that best practices for conducting serosurveys have often not been applied. There is a little comparability of laboratory testing.

Dr Mulders also discussed generating a framework for the national decision-makers, national programme managers and agencies/partners/donors based on the key questions to be answered. For the national decision-makers, it is important that they should know the relevance, process, costs and outcomes of the serosurvey. Validity of serosurveillance is also essential for the agencies, partners and donors. It was recommended to develop a set of best practices and guidelines for immunization serosurveillance and encourage standardized laboratory testing methodology for VPD serosurveillance.

(2) Experience of China laboratory network in conducting serosurvey

Mr Naiying Mao from China CDC presented on measles and rubella serosurveys in China. Provincial CDCs are responsible for the development and implementation of serosurvey programmes for measles, including quality control, analysis, evaluation and reporting of results. Prefecture CDC are responsible for implementation of the testing and technical support, and county-level CDCs are responsible for the collection and transport of specimens. The national measles laboratory is responsible for the evaluation of different testing methods (ELISA and hemagglutination inhibition assay) for measles antibody detection using plaque reduction neutralization test (PRNT). Since hemagglutination inhibition antibody has poor correlation with neutralization antibody, it is not recommended for serosurveys. A commercial quantitative ELISA, Huaguan, is being used for the serosurvey. The survey used stratified random sampling of children under one year old to >20 years old.

A serosurvey in Liaoning province, conducted from 2007 to 2012, showed that in 2012, among seven age groups, the positive rate of measles antibody was lowest in children under one year old (85.7%), followed by adults over 20 years (89.7%). A survey in Shaanxi province in 2011 indicated that the positive rate of measles antibody was lowest in the five- to six-year-old age group (82.5%). A survey conducted in Hunan province in 2012 indicated that the positive rate of measles antibody was lowest among children aged one to 1.4 years old (81.0%). These results imply that the susceptible groups for measles infection have shifted in recent years. Incidence of measles decreased in Chongqing City after SIAs were conducted in April 2009–2011. However, significant variations of the positive rate of measles antibody among different districts were noted. A decrease in measles cases in Gansu province was reported after the SIAs conducted in 2008 and 2010. In 2010, the positive rate of measles antibody was lowest (<60%) in Jingtai prefecture. Serum samples were collected from district and county levels. The results of the serosurvey varied among provinces, prefectures and counties due to different vaccination coverage rates. Along with the acceleration of measles elimination in China, the positive rate of measles antibody in health population in most provinces increased. However, only a few provinces achieved the positive rate of measles antibody recommended by WHO. Therefore, consecutive serosurveys of measles antibody in health population are critical for understanding the measles epidemic.

(3) Experience in Japan

Dr Makoto Takeda from NIID presented the serosurvey system adopted in Japan and explained how it has been used in the effective management of the immunization programme in
Japan. The serosurvey system in Japan is called the national epidemiological surveillance of vaccine-preventable diseases (NESVPD). They system, which was started in 1962, is conducted under the Infectious Disease Law of 1999. The aim of NESVPD is to predict prevalence of diseases comprehensively on a long-term basis by conducting investigations, to understand the actual situation of herd immunity, and to search for causative factors as well as to study various sources of epidemiological information. Dr Takeda discussed the procedures in conducting the serosurveillance programme. A particle agglutination test was used to measure measles IgG antibodies. A serosurvey was done in 2008 and a total of 7031 samples were tested among population aged zero to >70 years old. The results showed that among the cohort aged 8–17 years old, the antibody levels were lower compared to other aged groups. The percentage of samples that tested positive for the measles antibody was <85% at 1:64 titre.

During 1997–2005, a single dose of measles vaccination was given to children aged 12 months to 90 months. In June 2006, a five-year programme calling for two doses of MR vaccine was started; wherein, the first dose was given at one year of age and the second dose at age five or six years. However, this two-dose vaccination policy was too late or insufficient to contain the subsequent measles outbreak in Japan in 2007. A total of 11 005 measles cases were reported in 2008, with the majority of measles cases found among unvaccinated populations under one year old and between eight years and 20 years old. These data correlate with the serosurvey done in 2008, when the cohort aged eight years to 17 years old had low antibody titres. The results of the serosurvey in 2011 showed an increase in measles antibody titres in children older than two years old, especially in the cohort aged eight to 17 years old, where the percentage of samples that tested positive for the measles antibody was >90% at 1:64 titre. The five-year vaccination programme from 2006 resulted in the increase of antibody titres in the population. Also, the measles incidence has decreased from 11 105 cases in 2008 to 293 cases in 2012. However, rubella cases have increased in 2012 with 2353 rubella reported cases and as of February 2013, 1029 rubella cases were reported.

During 1977–1994, group immunization was conducted for female junior high students at school. From 1994–2006, rubella immunization was given to boys and girls aged 12–90 months, and individual immunization for male and female junior high students was done at hospitals. In 2006, a two-dose schedule of combined MR vaccine was adopted. During 2008–2012, supplementary immunization activities targeted adolescents aged 13–14 years old (or in junior high school) and teenagers aged 15–16 years old (or in third year high school). In the 2011 NESVPD for rubella, male adults aged 20–64 years old had lower antibody titre compared to females in the same age cohort; however, 80% of males had high antibody titres. Among the rubella cases reported in 2012, the highest number of reported cases were among males aged 17–50 years old, while females rubella cases were low in number and the most affected age groups were 17–30 years old and ≥50 years old.

2.2.8 Strengthening rubella and CRS surveillance and the role of the laboratory

(1) Improving global rubella virus surveillance

Dr Min-hsin Chen from US CDC presented the strategic plan for rubella and CRS surveillance, which covers the period 2012–2020, molecular techniques for rubella virus detection and some practical considerations to improve rubella virus surveillance. The global rubella goals include plans to achieve regional measles and rubella/CRS elimination by the end of 2015 and achieve measles and rubella elimination in at least five WHO regions by 2020. The strategy includes: (a) achieve high population immunity by providing high vaccination coverage with two doses of MR vaccine; (b) monitor disease using effective surveillance and evaluate
programmatic efforts to ensure progress; (c) develop and maintain outbreak response preparedness; (d) communicate and engage to build public confidence in and demand for immunization; and (e) perform the research and development needed to support cost-effective operations and improve vaccination and diagnostic tools.

Dr Chen discussed the importance of molecular testing in the diagnosis of rubella and CRS. Molecular techniques for detection of the virus can be used in providing classification of the cases within three days after rash onset, monitoring infectivity of CRS infants, distinguishing between vaccine and wild-type viruses, establishing endemic genotype baselines and genetic data that can be used to track transmission pathway and identify sources of infection. Other methods can also be used to recover the rubella virus from clinical specimens by using tissue culture; however, microscopic observation is not confirmatory because of the lack of visible cytopathic effect in clinical rubella cultures and either RT-PCR and/or immunofluorescent assay or immunocolorimetric assay are needed to confirm the virus isolated by culture. Urine is not ideal for virus isolation.

Diagnostic conventional RT-PCR or real-time RT-PCR and genotyping RT-PCR can be the methods of choice for rubella virus detection due to detection of the virus gene from clinical specimens (direct detection) or from infected tissue culture (virus isolate). Throat swab, oral fluids and urine are the most reliable specimens for detecting rubella virus by conventional RT-PCR and real-time PCR for CRS surveillance. US CDC developed the conventional diagnostic RT-PCR with three primers, and real-time RT-PCR for rubella has also adopted a three-primer RT-PCR protocol with sensitivity to detect 10 RNA copies. The modified, three-primer protocol (one forward and two reverse primers) showed improved sensitivity on detecting clade 2 viruses. To improve the sensitivity of rubella genotyping RT-PCR, two small overlapped fragments as sequencing templates to cover the 739-nt molecular window can be generated. Genotyping can also be done on alternate specimens such as serum and DBS. The best specimens are IgM-positive serum samples collected within four days of rash onset. Rubella molecular practice panels were distributed to four WHO regions including the Western Pacific Region in 2012, and most laboratories successfully completed real-time RT-PCR, genotyping RT-PCR and sequencing/genotyping. There are 116 countries reporting laboratory-confirmed rubella. Fifteen out of 108 countries were reporting genotype information in 2010, and 10 out of 96 countries were reporting genotype information in 2011. Rubella genotypes 1E, 1G and 2B had a wide geographic distribution and were frequently found; 1h and 1j were reported sporadically or in geographically restricted regions. Genotypes 1D, 1F, 1i, 2A and 2C were inactive during this period. Viruses of genotype 1C were found only in the Region of the Americas and were likely eliminated in 2005.

(2) Rubella and CRS surveillance in China

Dr Wenbo Xu from China CDC presented an update on rubella virus and CRS surveillance in China. MR and MMR vaccines were introduced into the national immunization programme in 2007. However, a rubella outbreak occurred in 2008 with an incidence of 9.1 per 100 000 population. In 2012, a total of 40 362 rubella cases were reported, and the incidence rate was 3.00 per 100 000 population. Compared to measles, the incidence of rubella was higher than in 2009. The epidemic pattern did not change between 2004 and 2012. During 2004–2012, rubella cases were reported throughout the year, and most of cases were seen between March and June, but a small peak also occurred in December. The high prevalence of rubella in China alternated between different regions and continuously occurred in areas covering eastern, central, and

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western portions of the country from 2007 to 2011. In 2012, the rubella incidence in six provinces decreased to <1 per 100 000 population. The proportion of reported rubella cases was high in children under 15 years of age. 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.

In the provincial laboratories, virus isolation and real-time RT-PCR are conducted in parallel. Then, if real-time PCR is positive, conventional RT-PCR and virus isolation are performed. Sequencing is also performed for RT-PCR-positive samples. Rubella isolates are transferred to China CDC for sequence determination/reconfirmation and genotyping. If provincial laboratories determine rubella virus sequences, sequence information is sent to China CDC. Sequencing results are sent to the WHO Regional Office on a monthly basis. During 1999–2012, 1662 rubella virus isolates were detected from 28 provinces. Of these, 953 rubella isolates were genotyped and four genotypes were identified: 1E (n=810), 1F (n=15), 2A (n=3), and 2B (n=125). Both 1E and 2B are in circulation in China. Genotype 1E rubella virus has been predominant since 2001 and was detected in 18 provinces of China in 2012. Genotype 2B was isolated in all provinces since it was first detected in 2001. The detection rate of genotype 2B increased from 11.8% in 2011 to 27.6% in 2012 and was detected in 10 provinces in 2012. It was observed that the genotype 2B virus detected in 2011 was different from genotype 2B detected in 2000 and 2008 (similarity: 95.9%). Genotype 2B virus in 2011 continues to circulate in the provinces, while new lineage 2B virus has emerged in 2012.

Dr Xu also presented the phylogenetic analysis of rubella 1E strains detected in China during 2001–2011 and 2B strains from 2000–2012. 1E strains could be divided into Cluster 1 and Cluster 2. Cluster 1 was predominant and may be unique to China and was continually imported into other countries since 2006. The most recent common ancestor of global genotype 1E rubella virus can be traced back to 1995 (from Europe). On the other hand, the 2B virus found in 2000 continuously circulated throughout 2008 (lineage 1). 2B strains from 2006 were related to importation. In 2008, several importations of 2B virus occurred and distribution was widespread in China (lineage 2 virus from 10 provinces; lineage 3 virus from Anhui province). Therefore, continuous rubella and CRS surveillance in China is necessary.

A three-year Ministry of Health–WHO project to establish prospective rubella and CRS surveillance was initiated in two cities in Shandong (Jinan and Yantai) and Heilongjiang (Qiqihaer and Haerbin) provinces in 2010. This project involved investigation and follow-up of pregnant women with suspected rubella and suspected CRS cases in close collaboration with the birth defect surveillance system. Full investigation of rubella outbreaks, the study of rubella vaccine efficacy as well as surveillance of rubella vaccine immunization coverage are being carried out. The results of this project showed that during 2010–2011 in Haerbin, six pregnant women with suspected rubella were reported. Of these, two cases were laboratory-confirmed (one case with two follow-up visits, gave birth to a normal infant) and two clinical cases (one pregnant woman had close contact with rubella case: classified as discarded case). In Yantai, one laboratory-confirmed case was detected in 2011 (one follow-up visit, had an abortion). During 2009–July 2012, a total of 735 suspected CRS cases were reported in project sites. Of these, three (0.41%) were laboratory-confirmed cases, 53 (7.21%) were clinical diagnosis cases, 652 (88.71%) were excluded cases, and 27 (3.67%) have pending results.
(3) Rubella and CRS surveillance in Japan

Dr Yoshio Mori from NIID Japan presented rubella and CRS surveillance in Japan. The national surveillance system of infectious diseases in Japan collects data on patients/cases and pathogens independently. For patient reporting, physicians who treat patients with infectious diseases, as defined by the law, must report them to local health centres. Then, the local health centres register the data through a computer network. Then, NIID sums up the case data, reports to the Ministry and WHO, and releases information to the public. On the other hand, for pathogen reporting, health centre staff can request physicians to collect and send specimens to prefectural public health laboratories. When the pathogens are identified in the laboratories, the results are registered and NIID sums up the pathogen data. In the case of rubella, physicians can report based on clinical or laboratory diagnosis. The rate of laboratory confirmation of rubella was 75% in 2012. Almost all laboratory diagnoses are based on detection of specific IgM.

Rubella cases have been reported since 1999, and until 2007, data were collected from sentinel site hospitals. Japan had a rubella epidemic in 2004, but the number of cases has decreased over the years, and only 87 cases were reported in 2010. However, a relatively large epidemic that occurred in 2012 (from Osaka to Tokyo), with more than 2000 rubella cases reported, is still ongoing as of February 2013. Also, CRS cases were reported from all hospitals. In non-rubella epidemic years, no more than two CRS cases per year were reported. In 2004, 10 CRS cases were reported accompanied by the rubella epidemic. Three CRC cases originated from other countries: India (2005), Philippines (2009) and Viet Nam (2011). In 2012, six CRS cases were reported. During the 2012 rubella outbreak, about 75% of patients were males, the majority of which were aged 20–40 years, while the majority of female patients were aged 20–29 years old. In contrast, very few cases were reported among children. It was explained that males aged 20–40 years old and females aged 20–29 years old have low rubella antibody titres because males over 30 years old had no chance to have rubella vaccination, and many females aged 20 years old did not have vaccination due to confusion by change of vaccination schedule. Among rubella patients in 2012, 90% had unknown or no vaccination history of rubella-containing vaccine and primary and secondary vaccine failure were estimated about 10%.

Between 2011 and 2013, many rubella viruses were detected and the majority were classified into genotypes 2B and 1E. In the rubella outbreak of 2004, it was thought that genotype 1j was the major virus circulating in Japan, but genotype 1j strains disappeared in 2012. A phylogenetic tree of the genotype 1E viruses showed that the viruses made distinct clusters, and that they are related to viruses detected in China and South-East Asia. A phylogenetic tree of genotype 2B strains suggested that the viruses found recently in Japan originated from several distinct sources. In order to eliminate rubella in Japan, it is important for adult males to be vaccinated for rubella.

(3) Rubella molecular surveillance in the United Kingdom

Dr Li Jin from HPA presented on rubella molecular surveillance in England and Wales. In 1969, selective rubella immunization policy (targeting teenage girls) was effective in reducing the number of CRS cases; however, susceptible women are still at risk from circulating rubella in children. In 1988, MMR was introduced as universal immunization to 12-month-old babies. During 1996–1997, there was an increase in cases of rubella mainly in university-age males not previously offered MMR or a rubella vaccine. Twenty-three cases of rubella in pregnancy were also reported. From 1997 to 2008, 13 congenital rubella births were registered with the National Congenital Rubella Surveillance Programme. Rubella is now rare; only <1% of notifications tested are confirmed. Data from 1971 to 2008 showed a significant decrease in CRS and rubella-associated terminations of pregnancy after the introduction of MMR vaccinations in 1988 and in 1996. Sampling, processing and testing of oral fluid-crevicular fluid was described. During
2005–2008, 34 cases tested rubella positive by PCR and genotyping was performed. Rubella strains were 1E and 1g (n=2) in 2005 with epidemic links from France and the Russian Federation; 2B, 1D and 1j (n=11) in 2006; 2B and 1g (n=13) in 2007; and 2B, 1E and 1a (n=8) in 2008 with epidemic links from Italy, Kazakhstan and Poland. Epidemic links of cases in 2006 and 2007 were unknown. In 2009, no positive case was detected. During 2010–2012, 34 cases were rubella positive. Rubella strains were 2B, 1E and 1a (n=7) with epidemic links from Switzerland and China; the epidemic link of genotype 2B in 2011 (n=2) could not be established. The genotypes 2B, 1g, 1B and 1a (n=25) in 2012 had epidemic links from the Russian Federation and maybe Romania.

Dr Jin outlined the assays developed by HPA to meet WHO recommendations for sequencing.7,8 Dr Jin highlighted that successful rubella diagnosis of suspected cases depends on the type of specimens collected (e.g. serum, throat swab, oral fluids), timing of collection, storage and shipment of specimens (for PCR, sample is stored and shipped at +4°C or -20°C). Data on the comparison of sensitivity of various samples such as urine, serum, blood, plasma, oral fluid, skin biopsy and nasopharyngeal aspirate from CRS cases were presented. The results showed that using secretion (e.g. nasopharyngeal aspirate) could be useful in the detection of rubella virus from children with CRS up to 12 months of age. A couple of HPA’s collaborative CRS projects with Sudan and India were highlighted.9,10 The need for laboratory confirmation to monitor rubella elimination is emphasized by poor sensitivity of the clinical case definition. Surveillance of rubella in England and Wales has included IgM testing of oral (crevicular) fluid from reported cases/patients since 1994. During 1999–2008, oral fluids from 11 709 (84%) of 13 952 reported cases were tested; 143 (1.0%) cases were confirmed. The enhanced oral fluid surveillance system has proven valuable for accurately assessing progress toward achieving the WHO goal of eliminating rubella and CRS.

2.2.9 Reports from global specialized laboratories and regional reference laboratories

(1) Report from NIID, Japan

Dr Katsuhiro Komase presented activities performed by NIID as the WHO measles GSL and RRL. Dr Komase conducted on-site reviews for Anhui and Hainan provincial laboratories in China from 29 August to 7 September 2012; attended the 5th Regional Hands-on Training Workshop on the Laboratory Diagnosis of Measles and Rubella focusing on Molecular Diagnosis from 24 October to 11 November 2012 at PHLC, Hong Kong (China) as a temporary adviser; and participated in the Workshop on Laboratory Diagnosis Techniques for the Control of Vaccine Preventable Diseases, including Poliomyelitis and Measles from 31 January to 3 February 2013, which was co-hosted by JICA and NIID.

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Based on the WHO definition of measles elimination, Japan is close to measles elimination; however, re-establishment of endemic measles transmission caused by imported measles virus poses a threat for the achievement of measles-free status in Japan. Thus, molecular analysis in routine case and outbreak investigations during the elimination phase of measles control is critical to document the genotype of each new cluster and demonstrate the absence of sustained transmission of one genotype. Since 2010, genotyping of circulating measles viruses in Japan has been performed by sequencing the 450 nucleotides of N gene (N-450) and analysed phylogenetically. In 2010, circulation of measles genotypes D4, D8, D9 and H1 was reported. Measles genotypes D4, D8 and D9 continued to circulate throughout 2011–2012. In 2011, a new genotype (G3) was detected, while H1 was not detected. In 2012, circulation of H1 was detected, whereas G3 was not detected. In summary, 162 measles virus strains were sequenced in Japan, no endemic strain was found to be circulating, and mixed genotypes detected were associated with importations.

Phylogenetic analysis of D4 and D9 genotypes with epidemiological data was conducted. The results revealed that genotype D4 was epidemiologically linked to Europe, India, New Zealand and Pakistan; however, lineage of measles virus strains from Europe could not be distinguished. Genotype D9 was epidemiologically linked to Cambodia, Indonesia, Malaysia, the Philippines, Singapore and Sri Lanka during 2010–2012, and D9 sequence results showed that three clusters of measles strains were detected from the Philippines. To identify further lineages of a single genotype, two D4 genotypes (epidemiologically linked to France and detected from Osaka and Kobe in 2011) and one D9 genotype (detected from Aichi in 2010) were compared with the same strains detected at different times. These three measles virus strains were inoculated into Vero/hSLAM and B95a cell lines (occasionally) and were sequenced using wider sequencing windows: N gene (1578 nts), H gene (1958 nts) and M gene (1008 nts). The data showed that the two D4 genotypes were identical, while the D9 genotype was only detected as identical using M gene sequence window. It was concluded that N-450 sequence window is not enough to distinguish differences in the strain and wider windows (N, M, H gene analysis) are also insufficient in some cases. Virus isolation is helpful to identify the differences in strains in more detail. In addition to molecular analysis, epidemiological investigation is equally important to confirm the origin of the virus.

(2) Report from VIDRL, Australia

Ms Vicki Stambos presented the activities of VIDRL as a WHO measles and rubella RRL. VIDRL has been preparing PT samples for global network laboratories, and conducting confirmatory testing for Fiji, New Zealand, Papua New Guinea and the Republic of Korea since 2011. The laboratory also received samples from New Zealand, Samoa and Solomon Islands for case investigations in 2012. VIDRL participates in two serology quality assurance programmes of the Royal College of Pathologists of Australasia: vaccine-preventable diseases module for measles testing (IgG/ IgM) conducted twice a year; and antenatal serology module for rubella testing (IgG/IgM) conducted four times a year. The Siemens kit is used in many laboratories in Australia for measles IgM testing, but other test kits such as Diesse ELISA, DiaSorin chemiluminescent immunoassay (ChLIA), MBL Bion immunofluorescence assay and in-house kits are also used. Most of the laboratories in Australia used BioMerieux enzyme-linked fluorescent immunoassay (ELFA) and Siemens EIA for measles IgG testing. For rubella IgM testing, BioMerieux ELFA, Siemens EIA, Abott chemiluminescent microparticle immunoassay (CMIA) and Abbott microplate enzyme immunoassay (MEIA) are used. The majority of laboratories use Abbott CMIA, Roche Diagnostics electrochemiluminescence assay and Siemens ChLIA for rubella IgG.
At VIDRL, Enzygnost anti-measles virus IgM EIA (Siemens) is used routinely, and Platelia measles virus IgM capture EIA (Bio-Rad) has been used as a confirmatory test since January 2011. For rubella serological diagnosis, Access anti-rubella virus IgM capture ChLIA (Beckman Coulter) is used routinely, while Rubella IgM capture EIA (DiaSorin) and Enzygnost anti-rubella virus IgM EIA (Siemens) are used for confirmatory testing. 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.

In 2011, 5913 samples were tested for measles IgG and 5682 for measles IgM; 751 samples were tested for rubella IgG and 289 for rubella IgM. In 2012, 6210 samples were tested for measles IgG and 5883 for measles IgM; 734 samples were tested for rubella IgG and 500 for rubella IgM. In 2011, among 31 measles IgM positive cases (including five IgM equivocal), 23 cases (74.19%) were also positive by PCR. Genotyping was done on nose and throat swab samples and for two cases on serum samples. Genotypes identified were D8 (n=18), D4 (n=8), D9 (n=3), H1 (n=1) and vaccine-related A (n=5). Genotypes could not be confirmed for three cases due to low virus titre. In 2012, among nine measles IgM positive cases (including one IgM equivocal), seven cases (77.78%) were PCR positive. Genotypes identified were D8 (n=6), D9 (n=2), B3 (n=2) and A (n=1). No case was reported in 2013 as of 21 February. Of the 18 samples received from Samoa, five samples were rubella IgM positive, but no sample was measles confirmed. Among 130 samples received from Solomon Islands, 52 (40%) samples were rubella IgM positive and seven (5.4%) samples were equivocal. There was no measles-confirmed case. Rubella genotype was 2B. The laboratory also received two serum samples collected four months apart from a pregnant woman with non-vesicular rash from New Zealand. The case was positive for both rubella IgM and IgG.

An evaluation of the rubella IgM kits was performed since rubella IgM kits have variable results. Three kits by Siemens, Nova Tec and Trinity Biotech were evaluated. Based on the preliminary results, the Siemens kit has high sensitivity (detected most of the positive samples), followed by the Nova Tec kit. For specificity, the Trinity Biotech kit gave non-specific reactions.

(3) Report from China CDC

Dr Zhang Yan presented on the activities of the RRL and national/subnational laboratories in China, and shared updates on the serological detection, virus surveillance and quality control of the national measles laboratory network. China CDC serves as the RRL and national laboratory for 31 subnational/provincial laboratories. One China CDC staff served as temporary adviser at the 5th Regional Hands-on Training Workshop on the Laboratory Diagnosis of Measles and Rubella, held in Hong Kong (China) from 24 October to 11 November 2012. In January 2013, one staff from China CDC participated in the on-site accreditation review of Cambodia’s national measles laboratory. Historically, China CDC has served as the reference laboratory for the Democratic Republic of Korea; however, in recent years, no sample was received. China CDC is responsible for distributing Vero/hSLAM cells to the provincial laboratories; evaluating and recommending kits for ELISA and molecular detection (real-time RT-PCR); recommending virus surveillance strategy, genotyping and data management; implementing the quality control system and providing technical support; providing PT panels (serology, practice panel for molecular detection) annually and reconfirmation; conducting training; and performing on-site accreditation (12–13 provinces) annually.
China has made a lot of progress towards measles elimination since 2009. Several SIAs were conducted in specific provinces from 2004 to 2009. A new low record of measles cases was noted in 2009, a 60% decrease in measles morbidity compared in 2008, and after the nationwide SIA in 2010, a 74% reduction of the cases was reported compared to 2009. In 2012, more than 38,000 serum samples were tested from suspected sporadic cases; of these, 17% were measles IgM positive and 13.28% were rubella IgM positive. This indicates that it is necessary to do the fever and rash syndrome surveillance to identify other pathogens. For suspected outbreaks, the proportion of samples for serology confirmation was very high (100%) in 2012, but the proportion of samples for virus isolation/genotyping was very low (40%), and this is a big challenge for China. From 2009 to 2012, surveillance and laboratory performance indicators improved. Performance indicators on suspected cases with adequate investigation, suspected cases with adequate blood specimens and percent of blood test results reported within seven days were more than 90% in 2012. Discarded measles rate increased to two per 100,000 population. However, collection of throat swab for virus isolation/PCR decreased in 2012. Measles genotype H1 virus is still endemic in China (n=449), with few cases of D9 (n=13), D8 (n=1) and A-vaccine strain (n=4) recorded in 2012.

China’s laboratory network participates in WHO on-site accreditation annually, and only one laboratory (Xinjiang subnational laboratory) is not accredited as of 2012. In July 2012, two rounds of training on molecular diagnosis of measles and rubella (real-time RT-PCR, FTA card and genotyping directly from throat swabs) were conducted in Beijing and were attended by more than 40 staff from 32 provincial laboratories and some prefecture laboratories. Another workshop was held in December 2012 in Beijing.

China’s measles and rubella laboratory network functions very well and can support cases diagnosis for verification of measles elimination, but some challenges still remain. Since early confirmation of suspected measles cases and timeliness of genotyping are very important for measles elimination, provincial laboratories were recommended to do genotyping directly from throat swabs and report the sequence to the national laboratory in a timely manner. The Chinese Government allocated 240 million Renminbi (equivalent to US$ 38.6 million) to support measles elimination projects, particularly to strengthen the capacity of provincial and prefecture measles network laboratories. With financial support from the Government, the responsibilities in different levels of the measles laboratory network were revised. Real-time RT-PCR was delegated to prefecture laboratories for case diagnosis, while genotyping will be performed in provincial laboratories with molecular testing capacity. The RRL/national laboratory will focus on data management and reporting, genotyping for measles isolates, quality control and accreditation for provincial laboratories, and provision of technical support to provincial laboratories. Since molecular detection was introduced to prefecture and provincial levels, quality assurance for molecular detection in province and prefecture levels is needed. Training on real-time RT-PCR will be conducted for provincial and prefecture laboratories, and the reporting system will be updated to include reporting of the molecular diagnosis.

(4) Report from PHLC, Hong Kong (China)

Dr Janice Lo from PHLC presented the MMR surveillance and laboratory activities of the RRL and national laboratory in Hong Kong (China). In 1967, a single-dose MCV was introduced, and in 1977, a single-dose RCV was introduced (for female primary schoolchildren). In 1991, MCV and RCV were replaced by MMR vaccine, which was given to children at one year of age. In 1996, a second dose of MMR vaccine was added and given to children at five years of age.
Measles became a notifiable disease in 1961, while rubella and mumps became notifiable diseases in 1994. Since 1978, CRS was voluntarily reportable to the Central Rubella Registry, but in 2008, it became a notifiable disease. Hong Kong (China) has made a lot of progress in measles control. A decrease in measles cases was noted in 2012, when only eight cases were reported. Of these, seven cases were laboratory-confirmed and one case was clinically confirmed. With these figures, if imported cases were disregarded, Hong Kong (China) has reached the elimination target. The situation with rubella is rather stable, as the disease is not very endemic in Hong Kong (China); however, 44 rubella cases were confirmed in 2012. Most of the mumps cases were clinically confirmed (n=151) since laboratory confirmation is not helpful. There was no detection of CRS cases for a long time in Hong Kong (China); however, in the beginning of 2012, three CRS cases were confirmed by rubella IgM test. It was later confirmed that these cases were imported from mainland China. Since then, no CRS case has been identified.

Measles and rubella laboratory testing is done upon request using IgM and PCR. In 2012, 515 clinical samples from 481 suspected measles cases were received. Two samples were taken from some of the cases, so the number of samples was more than the number of cases investigated. Of these, five cases were measles IgM positive. IgM-positive cases with recent vaccination were considered non-cases. PCR was performed if samples were collected very early after rash onset. A total of eight cases were notified. Timeliness of reporting measles results within seven days was 96.9%. In 2012, 1344 clinical samples from 1312 cases were investigated for rubella (rash illness and CRS). Among these, 44 were confirmed as rubella (20 cases were among cases with rash illness and 24 from cases without rash and CRS). Timeliness of reporting rubella results was 95.8%. Other tests are also performed on samples based on clinical information provided by clinician. These tests include direct detection/culture for enterovirus and serological testing for other agents: IgM/IgG for parvovirus B19, dengue, HIV, Epstein–Barr virus (EBV); and paired antibody titres for Mycoplasma pneumoniae, adenovirus, enterovirus, cytomegalovirus (CMV), and others.

During 2009–2012, 14 measles viruses including two vaccine strains and 17 rubella viruses were isolated; four mumps viruses were isolated in 2009 and 2011. Measles H1 strain, which is endemic in Hong Kong (China), circulated from 2009 (n=5) through 2012 (n=4). The laboratory detected additional strains in 2010: B3 (n=1), D8 (n=1) and D9 (n=1). Measles strains D8 and D9 were still detected in 2012. Eight cases of vaccine strain (A) were detected from 2009 to 2012. Rubella genotyping data showed that 1E strains were continuously detected from 2008 to 2012; 1j was only detected in 2010; and 2B strains were detected during 2009 and 2011–2012. Mumps genotypes B, F and G were detected in 2009, genotypes G and H were detected in 2011, genotype F was again detected in 2012. PHLC has been entering measles sequences from 2011 data into the MeaNS database since January 2013. Real-time RT-PCR for rubella detection in clinical specimens was adopted in January 2013. Evaluation of real-time RT-PCR for measles detection is in progress.

As a WHO measles RRL, this laboratory provides confirmatory testing and genotyping for other national laboratories in the Western Pacific Region and has hosted WHO hands-on training workshops. Genotyping using measles/rubella IgM positive or equivocal serum samples provided valuable background genotype information on circulating measles and rubella virus strains in the Region.
2.3.10 Mumps

(1) Mumps nomenclature and laboratory diagnosis

Dr Li Jin from HPA, United Kingdom presented an update on mumps nomenclature, laboratory diagnosis, global genotype distribution and surveillance of mumps in England and Wales. In 2005, genetic characterization of wild-type mumps strains was proposed, and preliminary standardization of the nomenclature was initially started.\textsuperscript{11} There was limited data from a few countries and genotype A-L was proposed. During the 7th Global Measles and Rubella LabNet Meeting in 2009, one of the recommendations made was to encourage mumps surveillance. Since then, isolates and sequence data submitted to GenBank/HPA have increased, and genotype M was proposed. Data on more than 500 strains from 39 countries are available. During the 2011 WHO Mumps Nomenclature Update Meeting, several recommendations were proposed. In June 2012, the final version of the proposal on updating mumps nomenclature was published in the WHO \textit{Weekly Epidemiological Report}.\textsuperscript{12} Updates to mumps nomenclature include: (a) naming mumps virus (MuV) as proposed for measles/rubella virus; (b) removing sub-genotypes, and reclassifying genotype E and M strains as C and K; (c) two vaccine strains are designated as genotype N; there are 12 genotypes designated A-N (namely: A, B, C, D, F, G, H, I, J, K, L, N) excluding (E and M); (d) criteria proposed for establishing a new genotype; (e) recommendations made to encourage mumps surveillance; (f) guidelines proposed for genotyping MuV; and (g) mumps sequence database and strain bank established at HPA, and the global distribution of MuV genotypes updated.

Currently, among 193 WHO Member States, only 39 (20.2\%) are reporting MuV genotypes. Global distribution of MuV genotypes that were reported from 2005 to 2011 was presented. In Western Europe, the predominant MuV genotype is G, with few cases of H, D, J and C. In the Western Pacific Region, MuV genotypes B, F, G and H were reported. Of the 193 Member States, 118 (61\%) are using mumps vaccine in their national immunization schedule. Countries of the WHO African Region and South-East Asian Region have not yet introduced mumps vaccination in their routine immunization schedules. In the United Kingdom, multiple genotypes were identified during 1996–2012, including genotypes C, D, F, G (G1–G7), H, J and K. The results revealed a diversity of strains consistent with co-circulation of multiple genotypes of mumps.

Surveillance on mumps in the United Kingdom previously relied on clinical reports via statutory notification system. Sero-surveillance has been implemented since late 1994, and oral fluid testing is offered to all notifications of MMR. Since 1996, when molecular epidemiology for MuV started, testing of oral fluid samples was also used for detection of mumps virus genome by PCR. In the absence of vaccination, annual incidences of mumps were within a range of 100–1000 per 100 000 of the general population, with an epidemic peak every two to five years in England and Wales. During 2005–2009, mumps outbreaks occurred in England and Wales, with 43 378 and 7662 laboratory-confirmed cases, respectively. From 2002 to 2006, the highest number of mumps cases was in the age group of 15–19 year olds, while 20–24 year olds were the most affected from 2007 to 2012. Correlation between mumps IgM detection from oral fluid depends on timing of collection of the sample post onset of the disease, and it was shown that IgM could be detected in oral fluid samples up to 28–30 days after onset.


The MMR vaccine with the Urabe strain of mumps was used in United Kingdom from 1988 to September 1992. As there was an associated risk with Urabe-containing MMR, an alternative MMR vaccine with a different strain of mumps virus (the Jeryl Lynn strain) was introduced in 1992. Observed and projected MMR coverage was more than 90% among 16- and 24-month-old children and around 88% for children 36 months old in 2011.

She concluded that current mumps diagnostic tests present a challenge in identifying mumps cases, especially in previously vaccinated individuals. Because of the lower positive rate of IgM testing in early collected samples or in cases with a history of vaccination or previous infection, detection of viral RNA in oral fluid, throat swab or urine by RT-PCR should be included as a diagnostic method in addition to IgM testing. Mumps elimination/eradication remains a worthy but very difficult goal.

(2) Mumps virus surveillance in China

Dr Aili Cui presented on mumps epidemiology and molecular surveillance of mumps virus in China. Wild-type mumps virus in China was first isolated and identified as a new genotype in 1995. Mumps surveillance started in 2001 and was incorporated into the National Diseases Reporting Information System in 2004. Mumps vaccine was introduced into the national immunization programme in 2007. There were 442 clinical samples from mumps-suspected cases from 20 provinces between 2001 and 2012. A total of 205 mumps viruses were obtained in the past 12 years and were genotyped. Of these, mumps virus genotypes F and G were identified. Genotype F has been the predominant virus, and has been detected in 21 provinces since 2001. Mumps virus genotype G virus was detected in Liaoning, Shannxi and Fujian provinces in 2011. Some provinces still have surveillance gaps. The phylogenetic tree of the Chinese mumps virus based on the 316 nucleotide sequence of the SH gene from 1995 to 2012 was presented. The diversity of nucleotides based on SH gene sequences increased from 1995 to 2012, as more mumps viruses are obtained. Multiple circulating lineages occurred throughout China.

The phylogenetic tree of the representative Chinese mumps virus based on the 1749 nucleotide sequence of the HN gene revealed multiple circulating lineages in the HN tree. The nucleotide sequence and predicted amino acid variation of the HN gene of genotype F mumps virus strains were 0–2.86% and 0–2.40%, respectively. For most genotype F strains, the nine potential glycosylation sites (N-X-T or N-X-S) remain, but one disappeared in two mumps virus strains and a new one occurred in one mumps virus strain. For genotype G, the diversity within this virus is 0–4.75% based on the SH gene. The phylogenetic tree of genotype G mumps virus in China in 2011 showed that the strains obtained from Fujian and Liaoning are the closest to the strain from the United Kingdom in 2003 (EU606236, Homology: 99%). The strains from Shannxi are the closest to the strain from Japan in 2005 (AB699705, Homology: 99%). Mumps virus surveillance should be strengthened to determine whether other mumps virus genotypes are endemic in China.

(3) Molecular testing for mumps in the United States of America

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 since 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
Dr Rota described the detection of mumps RNA by real-time RT-PCR with same configuration used in measles and rubella detection. Buccal or nasal swab, urine, oral fluid and serum can be used for real-time RT-PCR assay. In 2012, 982 cases were reported by 43 states in the United States of America, and 376 cases were reported in 2011. Of these, 89% were confirmed, 11% were probable and 36% were laboratory-confirmed. Among 60% of cases for which vaccination status was known, 82% were vaccinated with at least one does of MMR vaccine.

Dr Rota presented a comparison of the sensitivity of laboratory diagnostic methods from a well-characterized outbreak of mumps in New York City in 2009. From the outbreak, 296 serum samples were tested using the US CDC capture IgM EIA. The results, stratified by timing of serum collection and MMR vaccination status, revealed that the assay has the ability to detect mumps IgM in 69% of the cases that had not received MMR and if serum samples were collected after three days of onset. However, in mumps cases with one or two doses of MMR, timing of collection should be more than three days after onset since detection of IgM is lower in samples collected within 0–2 days after onset. Mumps IgM detection using the US CDC capture IgM assay was compared with two commercially available kits, and an immunofluorescent antibody assay on serum samples from 205 cases that were confirmed by virus isolation as a gold standard method. The positive sensitivity ratios varied: 52% for US CDC IgM capture assay, 12.5% for commercial indirect EIA, 29% for commercial IgM capture assay, and 15% for immunofluorescent assay.

The results of real-time RT-PCR using mumps SH gene as the target performed on original buccal swab samples showed 66% positive in samples collected up to two days after onset and 22% positive in samples collected more than three days after onset. Among buccal swab samples from 205 cases that were confirmed by virus isolation, real-time PCR targeting the N gene was able to detect 94% mumps-positive cases compared to 53% mumps-positive cases using the SH gene. Eight mumps genotypes (C, D, F, G, H, I, K, N) were detected from 2006 to 2012, and the most prevalent was G. The outbreak in 2009 and also in 2010 was caused by mumps genotype G.

Dr Rota recommended that laboratory testing should be done to confirm suspected outbreaks of mumps, but not to confirm every suspected case. Laboratory testing should only be conducted on suspected cases meeting a stringent case definition. Because of the limited utility of serological testing, laboratories with capacity for PCR should consider establishing the mumps real-time RT-PCR assay as a diagnostic method. He recommended collecting samples to establish a genetic baseline for wild-type mumps.

2.3.11 Additional topics and discussion

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

Dr Jasmine Kwong presented the experience of using confirmatory serum samples for genotyping. The genetic characterization of measles virus is based on sequence analysis of the 450 nucleotides coding for the 150 amino acids at the carboxyl terminus of the nucleoprotein (N-450). Currently, a two-step nested RT-PCR protocol to yield a better sensitivity is being used at PHLC. Before 2010, the HPA primers for RT-PCR were employed, but one of the drawbacks of these sets of primers is that the resultant amplicons are six nucleotides short in the 5’ end, resulting in incomplete genotyping window. Since 2011, the primers have been changed as suggested by US CDC, covering the whole genotyping window, which has improved the genotyping success rate. The success of genotyping depends very much on the detection of viral
genome in serum. The sensitivity of RT-PCR using serum samples was compared to the sensitivity of IgM. It was found that using serum samples collected during the first three days after rash onset, the RT-PCTR detection rate was 81% compared to 91.2% for IgM detection. As IgM develops, the sensitivity of RT-PCR decreases. By comparing the sensitivity of measles genome detection by RT-PCR in different samples, it was revealed that serum samples have a lower sensitivity (76.3%) than nasopahryngeal, throat and urine specimens (>95%). Also, serum sensitivity drops more quickly in the first seven days. Therefore, Dr Kwong concluded that despite the convenience of performing all tests (including IgM and genotyping) using one specimen, it would be better to obtain other specimens as well.

In Hong Kong (China), genotypes D8, D9 and H1 were detected among cases, and vaccine genotype A was detected in cases with rash associated with recent MMR vaccination. Measles genotyping using serum samples referred by the national laboratories in the Western Pacific Region was performed at PHLC. The detection rates varied greatly from country to country, ranging from 0% to 100%. These variations could be the result of differences in date of sample collection from onset of illness, storage conditions, and repeated freezing and thawing of samples before testing. She emphasized that detection of measles virus by RT-PCR was higher when the specimen was taken during the first four days after rash onset.

Rubella genotyping is not as satisfactory as that of measles. She explained that it was technically difficult to obtain a long rubella sequence window (739 nts) in E1 coding region (nts 8731-9469). A two-step nested RT-PCR for rubella genotyping is used, and in addition, a two-fragment system is used in the second round to obtain a long sequence. The success rate for rubella genotyping ranged from 35.7% to 100%. In some cases, only partial sequences were obtained, but genotypes could still be determined. The rubella virus detected in Hong Kong (China) was mostly genotype 1E and 2B. Rubella genotyping of serum samples referred by the national laboratories in the Western Pacific Region showed that positive rates varied among countries, ranging from 0% to 100%. But in general, the rate is lower when compared with that of measles virus.

Serum samples can be useful for measles genotyping when viral isolates are not available, and the best results can be obtained when the serum samples are collected within four days after rash onset. Clinical and epidemiological information should be obtained to develop baseline data for devising testing strategy.

(2) Use of avidity testing

Dr Min-Hsin Chen from US CDC presented on the use of IgG avidity in measles and rubella case classification in low-incidence settings. When the incidence of measles and rubella infection and CRS is low, the positive predictive value of specific IgM tests for confirming a recent infection is also low. False-positive rates have come close to true-positive rates because true-positive rates have declined to very low levels, while false-positive rates have been constant. Therefore, IgG avidity can help confirm possible false-positive IgM results in a low-incidence setting. However, avidity results are interpreted in combination with the following: clinical symptoms; epidemiological data including age, date of rash onset and serum collection, vaccination history; and available results from other assays such as IgM, IgG, plaque neutralization titre, and RT-PCR. The IgG avidity helps to distinguish acute from past infection. Avidity differences can be detected by using protein denaturants in the washing step of a measles/rubella IgG ELISA: low avidity antibodies are washed away, while high avidity antibodies remain bound. Case classification of measles and rubella using IgG avidity testing (using serum samples collected close to onset date) is shown in Table 2. The cutoff for measles high IgG avidity is 70%, while the cutoff for rubella high IgG avidity is 30%.
Table 2. Case classification of measles and rubella using IgG avidity

<table>
<thead>
<tr>
<th>IgG avidity</th>
<th>Course of infection</th>
<th>Measles case classification</th>
<th>Rubella case classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (≤ 30%)</td>
<td>Primary exposure (within 3 months)</td>
<td>- Recent measles infection/vaccination; - Primary vaccine failures</td>
<td>- Recent rubella infection/vaccination; - CRS</td>
</tr>
<tr>
<td>High</td>
<td>Distant infection (rubella: &gt; 3 months; Measles: &gt; 6 months)</td>
<td>- Modified measles cases due to secondary vaccine failures - likely false IgM</td>
<td>- likely false IgM or prolong IgM response</td>
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Fully validated commercial measles and rubella avidity kit assays are available to complement current assays. Commercial kits for rubella avidity have been compared, and the results have been published.\(^\text{13}\) Rubella avidity testing can be used to differentiate recent and distant exposure of rubella virus. In elimination settings, IgG avidity assays can complement existing diagnostic tools in confirming unvaccinated acute cases and, in conjunction with adequate clinical and epidemiologic investigation, aid in the classification of vaccine failure.

(3) Use of conventional and real-time RT-PCR for case classification

Dr Paul Rota from US CDC led a discussion among the participants. He emphasized that laboratorians in the Region are responsible for orchestrating the use of all the tools available for case classification. It is necessary that the laboratories in the Region have a good understanding of the strengths and weaknesses of the tests. Dr Rota requested the participants to share their experiences with and opinions on integrating different testing techniques. It was noted that training workshops on using molecular techniques in the diagnosis of measles and rubella were being implemented in the Region and have been very successful. It was also brought up that laboratories in the Region are very good at generating sequence windows and getting PCR products for sequencing. Significant records of measles genotype data are submitted; however, there is a need to improve on rubella genotype detection. It is very important for the laboratories to know how real-time RT-PCR results can be used to help classify cases and to interpret the results. He emphasized that although the PCR technique is a powerful tool, laboratories should ensure quality control is in place.

(4) Summary of challenges of measles and rubella laboratories

<table>
<thead>
<tr>
<th>Country</th>
<th>Challenges</th>
</tr>
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<tbody>
<tr>
<td>Cambodia</td>
<td>- Quality of some samples is poor (e.g. haemolysis and DBS)</td>
</tr>
<tr>
<td>Fiji</td>
<td>- Need of consistency of measles awareness to clinicians and health personnel (diagnosis, reporting and follow up of cases) - Network of 19 subdivisional hospitals and three divisional</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Country</th>
<th>Issues and Actions</th>
</tr>
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</table>
| Lao People’s Democratic Republic | • Improve data management and coordination with other units  
• Provincial and district staff require refresher training on field investigation and collection of blood specimens  
• In-house control samples for measles and rubella for quality assurance in progress  
• Strengthen laboratory biosafety  
• Procurement of kit (ensure it is made on timely basis: three months in advance and keep 6 month stock at all times) |
| Malaysia                        | • Inadequate clinical/epidemiological information for test interpretation and further testing  
• Obtaining second sample for equivocal cases is not always successful |
| Mongolia                        | • Improvement of molecular epidemiological surveillance and sample collection from rash and fever patients in provinces (aimag) and rural districts (soum)  
• Introduction of CRS laboratory surveillance  
• Provision of required equipment and diagnostic reagents |
| New Zealand                     | • Serology and RT-PCR/virus isolation samples are not always received.  
• Low-level positive RT-PCR results, usually with a history of recent vaccination, can be difficult to distinguish from clinical disease.  
• Positive results are reported from other laboratories, but samples are not sent to the national measles laboratory for validation. |
| Papua New Guinea                | • Improve sample collection districts (only 10% reported cases in NHIS investigated in 2011)  
• Increase number of DBS samples for testing/retesting  
• Improve collection of throat swabs for rubella genotyping  
• National and provincial biosafety training planned in 2013  
• Strengthen PCR capacity in VPD surveillance - facility for HIV in place and can be shared |
| Philippines                     | • Collection and transport of serum and virus isolation samples in a timely (received by RITM within 72 hours from collection) and efficient manner  
• Equipment upgrade (current ELISA washer in use is a phased-out model)  
• Regions with high incidence of measles failed to collect samples for virus isolation |
| Republic of Korea | • Need to strengthen staff activity in PHERIs for measles and rubella diagnosis  
• Lack of staff in measles, mumps and rubella laboratory  
• Hard to obtain good-quality samples for virus detection (elimination status) |
| Singapore | • No laboratory confirmation on suspected cases  
• Some laboratory-confirmed cases were tested by non-national measles laboratory or non-WHO-accredited laboratories  
• Better coordination between laboratory and EPI |
| Viet Nam, North | • Lack of samples for measles/rubella viral isolation  
• Contamination when using CRS sample for isolation  
• Lack of real-time PCR (ABI) |
| Viet Nam, South | • Need to train staff from provinces on specimen collection including throat swab  
• Fever and rash surveillance is not at the expected level. Number of report and laboratory samples remains low.  
• Receiving throat swabs from provinces is rare |

2.3 **Japanese Encephalitis Laboratory Network**

**Opening session**

Dr Youngmee Jee, Regional EPI Laboratory Coordinator, welcomed all participants and advisers. She acknowledged the contribution of the laboratory networks during the last few years and emphasized the critical role of the Japanese encephalitis laboratory network to control JE in this Region.

2.3.1 **Overview of Japanese encephalitis (JE) control in the Western Pacific Region**

(1) **JE/acute encephalitis syndrome (AES) surveillance and JE vaccination in the Region**

Dr Kimberley Fox, Technical Officer, EPI, WHO Regional Office for the Western Pacific, presented an overview of the current status of JE surveillance and the vaccination programme in the Western Pacific Region. JE is the most common type of encephalitis in Asia. It is transmitted to humans primarily by the bite of a culex mosquito, with wading birds as the primary reservoir, but pigs serve as an amplifying host. JE/AES is primarily a disease of children under 15 years old (in endemic areas), which has an important implication on the surveillance and vaccination interventions. She emphasized that cases that manifest as encephalitis represent only a small portion of the total infection of the JE virus, and for every single case of encephalitis, there are 250–500 cases of mild febrile illness or asymptomatic infection.

Dr Fox discussed the objectives of surveillance before and after vaccine introduction, the WHO case definition and classification of AES, and the global WHO surveillance network for invasive bacterial vaccine-preventable diseases (IB-VPD). She also discussed the pros and cons of establishing a sentinel surveillance system for AES. It was noted that in the Western Pacific
Region, few countries have integrated JE surveillance and IBD-VPD surveillance. However, the primary focus of IB-VPD meningitis surveillance is on children under five years old. But because of the significant overlap between meningitis and encephalitis symptoms, and difficulty in distinguishing the same in children under five, it is convenient in many situations to integrate JE and VPD bacterial meningitis surveillance, resulting in meningitis-encephalitis surveillance. However, the successful integration of JE and IB-VPD surveillance depends on the specific surveillance system of the country, the sentinel sites being used, and the specimen management issues.

She highlighted the progress in JE surveillance in the Western Pacific Region. In countries with limited data on JE disease burden (e.g. Lao People's Democratic Republic, Papua New Guinea, Philippines), comprehensive AES aggregate reporting and sentinel hospital surveillance with laboratory confirmation have been established to assess disease burden and to characterize disease distribution. In countries with demonstrated disease burden and pilot vaccination (e.g. Cambodia), the purpose of expanding sentinel surveillance is to further demonstrate geographic range of JE and collect baseline data for measuring vaccine impact. In countries with established vaccination programmes (e.g. China, Malaysia, Viet Nam), the purpose of continuation of meningitis-encephalitis sentinel surveillance is to monitor vaccine impact, identify areas needing improved vaccine coverage, and detect areas with new disease transmission. WHO support for JE surveillance includes the development of generic case investigations forms, surveillance and laboratory database for and establishment of the WHO JE laboratory network in the Western Pacific Region.

Findings from the integrated meningitis-encephalitis surveillance (suspected meningitis/encephalitis cases due to Japanese encephalitis) from 2009 to mid-2012 showed that across countries (i.e. Cambodia, Philippines and Viet Nam), 13–21% of suspected meningitis/encephalitis cases are JE. The overall global estimate of JE burden is 67 900 JE cases annually (incidence 1.8 per 100 000), with 51 000 (75%) cases in children aged 0–14 years (incidence 5.4 per 100 000); however, only 10% of cases are reported to WHO.

Dr Fox outlined the substantial progress made in the initiation or expansion of JE vaccination programmes in the Western Pacific Region. JE vaccine has been introduced in eight countries with JE risk areas, including partial introduction in one country. For countries newly introducing JE vaccination, WHO recommends a catch-up campaign for a locally defined, broad age group using child health days or other multi-antigen campaigns, and integration into routine immunization schedule. Viet Nam has continued to expand the population covered by JE vaccination. Cambodia initiated pilot JE vaccination with live attenuated SA 14-14-2 vaccine manufactured by Chengdu Institute of Biological Products (CDIBP, China) for infants 9–10 months old in three provinces, and a campaign was conducted in February to March 2013. The Lao People's Democratic Republic conducted a campaign for children ages 12 months to under 15 years with live attenuated SA 14-14-2 vaccine in six northern provinces in March 2013.

During the fifth JE biregional meeting in 2011, key issues on JE surveillance were identified, including some critical variables that are difficult to obtain, most importantly: vaccination status; quality of data is not well monitored; surveillance indicators are not implemented in most places; coordination among multiple groups (epidemiology, laboratory, immunization) is necessary to effectively implement surveillance and to use results; JE case investigation is critical to understand reasons for continued cases in the presence of established JE vaccination programme (vaccine failure vs. failure to vaccinate, or disease in new areas).
Dr Fox highlighted the country action plans during the fifth JE biregional meeting in 2011. JE vaccination programmes still face real constraints in terms of vaccine supply and financing.

(2) Progress of the regional JE laboratory network

Dr Youngmee Jee presented the progress and challenges of the WHO JE laboratory network in the Western Pacific Region. The laboratory network was built on the model of the polio and measles VPD laboratory networks, based on EPI TAG recommendation in 2008 with support from PATH and the Korea CDC. The regional JE laboratory network consists of one GSL, two RRLs, and seven national laboratories. Most JE network laboratories were designated in the national public health institute, where laboratory testing for measles and rubella is also performed (i.e. Cambodia, Lao People’s Democratic Republic, Philippines, Papua New Guinea). The Papua New Guinea laboratory was the last of 10 laboratories to join the regional network in 2011 and started testing in 2012. Designation of 10 subnational JE laboratories in China (Chongqing, Guangdong, Guangxi, Guizhou, Henan, Shandong, Shanghai, Sichuan, Yunnan, Zhejiang) is ongoing. Procedures are being standardized (WHO manual), and in-house assays and commercial kits are being evaluated by US CDC and NIID, Japan. Three JE hands-on training workshops were conducted in 2009, 2010 and 2011 to build regional capacity for JE testing, and regional JE meetings were held in 2008, 2009 and 2011. Quality assurance mechanisms (proficiency and confirmatory testing) and WHO accreditation (using checklist) are in place. Case-based and aggregate data are received at the Regional Office for the Western Pacific on a monthly basis.

Most JE network laboratories are using commercial kits such as Panbio JE-Dengue IgM Combo ELISA; China CDC uses locally produced JE commercial kits (Beixi). Three JE network laboratories in the Region, namely NIID (Japan), NIHE (northern Viet Nam) and Pasteur Institute (southern Viet Nam) use in-house assays. The in-house assay used by NIHE is licensed in Viet Nam. Other techniques such as RT-PCR, virus isolation, hemagglutination inhibition test and PRNT are also being employed by JE laboratories in China, Japan, Malaysia, the Republic of Korea and Viet Nam.

Regional data from JE laboratories from 2009 to 2012 were presented. Cambodia had a JE IgM positive rate of 19–31%, and it was noted that there was an increase of dengue IgM positive cases in 2012. Two peaks of JE activity in the months of June–August and October–November were prominent in Cambodia. Japan had very limited data since JE is almost eliminated in this country and only positive cases were referred to the laboratory. The Republic of Korea had the highest number of samples tested, and from these, the JE IgM positive rate was 2–23% (2009 to 2012). Also, it was noted that there was an outbreak of JE in 2010 with relatively high positive cases (23%). There was limited data from Lao People's Democratic Republic, and most of the reported cases were from an outbreak; however, the JE IgM positive rate was 12–53%. The data also showed an increase of JE incidence during the period June–August in the Lao People's Democratic Republic. In Malaysia, there was a low detection of JE IgM with a positive rate of 3–8%. In 2010, while the JE IgM positive rate was only 3%, an increase in dengue IgM positive (9%) encephalitis cases was noted in Malaysia. Papua New Guinea only started testing in 2012. Seventeen samples (11 serum samples and six CSF samples) from eight cases were tested, and they were all negative. The JE IgM positive rate in the Philippines was 12–19%, and an increase in the detection of dengue IgM was noted in 2010 (15%) from suspected cases. The JE IgM positive rate in northern Viet Nam was 6–11%, while southern Viet Nam had a positive rate of 9–14%. A seasonal pattern of JE prevalence could not be determined in some countries since only a few sites were reporting cases and a few samples were collected and tested.
Quality assurance of national JE laboratories is documented through PT, confirmatory testing by RRL and on-site reviews of the laboratories for accreditation. In summary, Dr Jee said that network laboratories in the Western Pacific Region passed WHO PT from 2009 to 2012, and concordance rates from confirmatory testing improved during that period. Eight out of 10 laboratories are accredited. Also, 10–30% of AES cases from 2009 to 2012 were confirmed as JE, with a significant proportion of cases being over 15 years old. There is evidence for a need to introduce JE vaccine in Cambodia, the Lao People's Democratic Republic and the Philippines. She concluded that other etiologies of AES (both viral and bacterial) need to be investigated, and that countries should ultimately take responsibility for supporting and integrating JE surveillance/laboratory with other VPD surveillance programmes. Recommendations during the third JE laboratory network meeting held in Vientiane, Lao People's Democratic Republic on 30 May–1 June 2011 were reviewed. The JE laboratory network is still facing a lot of challenges such as: (a) JE laboratories do not fully collaborate with national EPI since JE surveillance is not fully established at the national level and JE vaccination is not available in some countries; (b) there is no global initiative since JE is prevalent mainly in the WHO South-East Asia Region and Western Pacific Region; (c) JE testing is also performed at non-WHO network laboratories (commercial or hospital laboratories), thus, JE laboratory testing data at the national level are difficult to capture; (d) incomplete information of cases including vaccination history and onset date; and (e) unstable funding for JE surveillance and laboratory network.

2.3.2 Quality assurance for JE laboratory

(1) Analysis of results of the 2011–2012 JE proficiency test

Dr Barbara W Johnson, Division of Vector-Borne Diseases, US CDC presented an analysis of the results of the 2011 and 2012 JE proficiency tests as part of the quality assurance programme for JE laboratory. Preparation and composition of the 2011 and 2012 proficiency panels were discussed. Laboratories were instructed to include JE IgM positive in-house controls during testing, and the worksheet should include: raw data, validity calculations, calculated results and interpretations of the results. For the 2011 PT panel, seven laboratories used Panbio JE-Dengue IgM Combo ELISA kit, four laboratories used JE and DEN IgM in-house assay, and three laboratories used Beixi kit (JE IgM only). It was noted that all laboratories scored over 80%. Dr Johnson highlighted a few deficiencies in the submission of results. For example, Papua New Guinea's JE calibrator optical densities (ODs) were high, resulting in false negatives; thus, the PT score was 89%. Mahosot Hospital laboratory in the Lao Democratic People's Republic had a typographical error in the calibration factor, and after correction, the PT score was 100% instead of 89%. Though the laboratories were asked to include in-house control during testing of the PT samples, not all laboratories included it due to non-availability of JE IgM positive samples. In 2012, four PT panel samples were distributed to 13 JE laboratories. Nine laboratories used Panbio JE-Dengue Combo IgM ELISA, three laboratories used JE and DEN IgM in-house assay, one laboratory used Beixi kit (JE IgM), and two laboratories used two assays (in-house and Panbio). Among the 13 laboratories that performed 2012 JE PT, 12 laboratories scored over 92%. The JE laboratory network is still facing some challenges. First, there is a lack of use of in-house control, which could detect problems with degraded kit reagents and problems with the washer/reader. Second, domestic and international shipping costs for the transport of specimens are on the rise.

Future directions for the JE laboratory network were presented. The 2013 PT panel will be distributed at the end of the JE hands-on training workshop in September 2013. The laboratories are encouraged to perform differential JE-dengue diagnosis to increase accuracy of estimation of
JE burden. There should be a trial use of DBS to facilitate shipping of PT panel. It is recommended that all the laboratories use in-house control and submit the trend in titre along with the PT panel result. WHO and US CDC will provide in-house controls to laboratories without JE IgM positive samples and also technical support to laboratories with less than 80% PT score.

(2) Confirmatory testing and accreditation of JE laboratories in the Western Pacific Region

Dr Youngmee Jee presented on the procedures of confirmatory testing and accreditation of JE laboratories in the Western Pacific Region. The national JE laboratories are required to send samples of CSF and serum to the RRL assigned to them for confirmatory testing. Confirmatory testing of samples from Cambodia, the Lao People's Democratic Republic and Viet Nam are performed in NIID, Japan. Samples from Malaysia and the Philippines are sent to Korea CDC. China CDC provides confirmatory testing for the provincial laboratories. The minimum sample volume required for CSF is 100-200 microliter (ul) and 250ul for serum samples. Confirmatory testing should be performed at least once a year, and before sending samples, national JE laboratories should contact the WHO regional laboratory coordinator to select samples to be sent to RRL or GSL. Results of confirmatory testing showed some discrepancies when different kits were used (e.g. NIID in-house assay versus Panbio kits). All laboratories had concordance rates of more than 90% in the late round of confirmatory testing. An accreditation programme was also designed for the JE laboratory network. Two separate WHO checklists were prepared for the RRLs and national laboratories. Eight of the 10 national JE laboratories were accredited, while two laboratories have pending accreditation. This accreditation programme is very helpful in identifying areas in the laboratory that require strengthening and also helps the laboratory get the attention of the ministry of health and other agencies in the JE programme.

2.3.3 Country reports

(1) Cambodia

Dr Buth Sokhal, Deputy Director of NIPH, presented the JE sentinel site surveillance and JE vaccination programme in Cambodia. The JE virus was first isolated in Cambodia in 1965 from mosquitoes. From 1990 to 2005, several research projects were conducted in four hospitals targeting hospitalized cases with meningitis-encephalitis, showing 18–31% of cases were due to JE. From 2006 to 2008, acute encephalitis/hospital-based JE sentinel surveillance was initiated in six provinces through a collaborative study of the national immunization programme, NIPH, PATH, WHO and Pasteur Institute. The results showed that 19% of meningoencephalitis cases were due to JE. Samples of CSF, acute and convalescent serum samples were collected from patients and sent to NIPH on a weekly basis and tested with Panbio JE-Dengue IgM Combo ELISA kits. Results were reported to the national immunization programme and to the Ministry of Health. Laboratory data from 2010 to 2012 revealed that the number of samples taken from meningoencephalitis cases and tested increased from 326 samples (121 CSF, 121 acute serum and 84 convalescent serum) in 2010 to 698 samples (266 CSF, 256 acute serum and 176 convalescent serum) in 2012. However, the JE positive rate decreased from 31.45% in 2010 to 21.64% in 2012. Age distribution of meningoencephalitis cases showed that the 6–10 year old age group represented 37% of cases and the 1–5 year old age group represented 27% of cases with high JE incidence. NIPH implemented quality assurance measures such as the use of in-house control, annual WHO PT, and confirmatory testing at RRL twice a year. Recent PT test scores and confirmatory testing concordance rates were 100%.
JE vaccine was introduced into the routine immunization programme in a few provinces in October 2009 using live attenuated SA 14-14-2 JE vaccine manufactured by the Chengdu Institute of Biological Products in China, and administered in a single dose one month after measles vaccination targeting children aged 10–24 months old. However, JE vaccine is not yet pre-qualified by WHO, and there are no financial resources for maintaining and scaling up JE immunization activities. Dr Sokhal discussed remaining challenges in the surveillance system. The laboratories in sentinel sites need to be strengthened for bacteriology testing for identifying bacterial causes of meningitis. Quality and volume of samples, sample packaging and transportation need to be improved.

(2) Lao People's Democratic Republic

(a) National Centre for Laboratory and Epidemiology (NCLE)

Dr Darouny Phonekeo from NCLE presented the JE laboratory and immunization activities in the Lao People's Democratic Republic. It was noted that AES is a notifiable disease under the National Surveillance System of Notifiable Selected Diseases. During 1989–1990, five JE IgM-positive cases were identified among 26 acute encephalitis patients from Mahosot and Setthathirat Hospitals. In 1992, among samples collected from 141 patients aged 21–30 years old in Mahosot Hospital, as well as samples from NCLE and the blood bank, 75% were JE-positive. In 1995, analysis of 141 serum samples collected from Khammouane Province (central), using the neutralization assay, indicated 50% positivity for JE antibodies in the 31–40 year old population. NCLE began JE testing after the first regional training in 2009, but the number of JE samples received and tested was relatively small. A total of 140 samples were tested at NCLE in 2010 (n=49), 2011 (n=44) and 2012 (n=47). Of these, 72 (51%) were JE IgM positive with seasonality from June to August. Confirmed JE cases were found in children under 15 years of age. However in 2011, 31% of confirmed cases were more than 15 years of age. Almost all samples tested at NCLE are received from central and northern provinces (Houaphan, 33; Oudomxay, 44 samples). Absence of reports in northern provinces might indicate weak surveillance rather than no transmission.

The first JE vaccination in the Lao People’s Democratic Republic will be introduced on 4–15 March 2013. It will target children aged one to 15 years old, and approximately 620 000 children will be immunized. JE vaccination will be introduced in six northern provinces where there is an evidence of high transmission. Live attenuated vaccine SA 14-14-2 was produced and donated by Chengdu Institute of Biological Products in China in collaboration with PATH.

The NCLE laboratory has participated in WHO quality assurance programmes since 2009. The laboratory’s PT panel score was 100% in 2012. Specimens for confirmation have been sent to NIID on a regular basis, and the most recent confirmatory testing concordance rate was 100%. WHO accreditation is pending, but WHO recommendations from the last visit are being implemented. Some challenges and requests were presented. The number of skilled staff to fulfil all laboratory demands is inadequate. Furthermore, the number of specimens received at NCLE is low compared with the number of AES cases reported to the Epidemiology Section. Provincial and district staff require more training on field collection of blood specimens, and staff need more technical and data management training. Mahosot Hospital continued to receive JE samples from provinces and shared JE testing data with NCLE and the WHO Regional Office for the Western Pacific.
(b) Mahosot Hospital

Dr Chansay Pathammavong from Mahosot Hospital presented the results of a study that examined central nervous system (CNS) infections and JE virus in the Lao People’s Democratic Republic. The study examined the testing of samples of suspected JE cases admitted to Mahosot Hospital, Vientiane from 2003 to 2011. Other hospitals involved in the study sent CSF samples to Mahosot Hospital for testing. Few provinces also conducted non-malaria febrile illness studies. Three methods of testing were used, namely, serology (Panbio JE-Dengue IgM Combo ELISA), JE cell culture on vero cells (from African green monkey kidney) and PCR of pan-flavivirus from Moureau et al. 14 Marseille Laboratory in France is developing a specific PCR system for JE. Interim results of the CNS infections study, from January 2003 to August 2011, showed that among 1073 patients with lumbar puncture, 544 (51%) cases had JE culture, and PCR was performed on 909 (85%) cases using CSF samples. Only one (0.2%) of 544 cases was JE culture positive, and four (0.4%) of 909 cases were JE PCR positive. Among 633 AES cases, 562 (89%) had CSF samples, 451 (71%) had acute serum samples and 258 (41%) had convalescent serum samples. A serological test done on CSF and serum samples showed that 97 AES cases (15%) were due to JE. The highest admission rate of AES-confirmed cases was from June to August during rainy season. The age group with the highest proportion of AES and JE cases was children less than 15 years old.

Other studies were also conducted. In one study, the first JE virus genome sequencing in the Lao People's Democratic Republic revealed that genotype 1 is present (Aubry et al. 2013). In another study, it was found that JE virus is the cause of 6% of fevers in patients presenting in rural areas without encephalitis (Mayxay et al. submitted). A third study showed that JE virus is a cause of AES in central, northern and southern Lao People's Democratic Republic. The study suggested that the major cause of AES is other agents and this need to be investigated.

The laboratory in Mahosot Hospital also participated in the WHO PT as part of the quality assurance programme. The PT score in 2011 was 100%; however, in 2012, PT score dropped to 70%, which is less than the 80% performance indicator required for WHO accreditation.

(3) Malaysia

Ms Norizah Ismail from NPHL presented JE surveillance and JE vaccination activities in Malaysia. The first JE case was confirmed in 1952 in Malaysia. Viral encephalitis has been a notifiable disease since 1988 (syndromic-based surveillance). There was an increase in JE cases detected following a Nipah/JE outbreak in early 2000. The JE vaccination programme commenced in 2002 in Sarawak. From 2007 to 2011, Institute of Medical Research (IMR) was designated the national JE reference laboratory; however, the function was transferred to NPHL in 2012. Ms Ismail explained NPHL’s algorithm of testing for JE. The diagnostic methods used include JE IgM capture ELISA, viral isolation and PCR. NHPL has been accredited as Malaysian Standards (MS) ISO 9001:2008 and is undergoing ISO 15189 certification. The laboratory also participates in external quality assurance programmes conducted by the Royal College of Pathologists of Australasia (molecular alphavirus/flavivirus) and WHO. Recent WHO PT score and confirmatory concordance rate were 100%. In-house control in graphic display is being implemented. From 2009 to 2012, around 4%-6% of samples tested in this laboratory were JE

IgM positive. Few JE confirmed cases were detected during 2009–2012. However, an increasing number of JE cases were detected in Selangor during 2010–2011 and in Sabah in 2012.

JE vaccination has been part of the national immunization programme in Sarawak since 2002 and is also conducted in peninsular Malaysia and Sabah within a two-kilometre radius of any JE case. In 2012, immunization coverage for JE (first dose) in Sarawak was 100%. From 2003 to 2012, the JE incidence rate decreased.

(4) Papua New Guinea

Ms Janlyn Kumbu from CPHL presented on JE epidemiology and laboratory activities in Papua New Guinea. In 1995, JE antibodies were detected among the inhabitants of Irian Jaya (land border with Indonesia). JE was first reported among inhabitants of Western Province (water border with Australia) between 1989 and 1998, and JE virus was isolated from mosquitoes in 1997. In 2003, an outbreak of JE was suspected in Milne Bay province but was not confirmed. A confirmed case of JE was reported from the Port Moresby area (national capital district) in 2004, and in 2010, a study from Port Moresby General Hospital confirmed JE as one of the causes of febrile encephalopathy in children.

CPHL is designated as a WHO national JE laboratory. Port Moresby General Hospital started surveillance in 2012, and national surveillance guidelines have been developed. JE diagnosis is performed using Panbio JE-Dengue IgM Combo ELISA supplied by WHO. As part of the quality assurance programme, the laboratory participates in WHO PT and confirmatory testing and uses in-house control. In 2012 and 2013 (February), 14 cases and 1 case were tested, respectively, for JE IgM and all samples were negative. Ms Kumbu highlighted some challenges. Few cases are reported since sentinel sites are not reporting. Obtaining adequate CSF samples is a challenge. Transportation of samples from provinces under appropriate conditions needs to be addressed. All surveillance unit staff recruited in 2012 and CPHL staff need training. Surveillance should be strengthened.

(6) Philippines

Dr Amado O Tandoc III from RITM presented updates on AES surveillance in the Philippines. Limited data exist on JE infection in the Philippines. In 2005, a study was conducted in San Lazaro Hospital showing six (40%) of 15 cases of encephalitis were due to JE. From 2009 to 2011, WHO funded a RITM study on meningitis, meningoencephalitis and encephalitis (MEMe) in five sentinel hospitals in Bulacan, Tarlac, Quezon City, Bicol and Iloilo. CSF and acute serum and convalescent serum samples were collected from suspected cases. Diagnostic methods used were serology (Panbio JE-Dengue IgM combo ELISA) and real-time PCR (US CDC protocol) for three bacterial pathogens. A total of 243 suspected cases were reported in this study. The MEMe study revealed that as many as 40% of cases were due to JE virus infection. Other pathogens were also detected (dengue, Haemophilus influenzae type B, Streptococcus pneumoniae and Neisseria meningitidis). Dengue is predominant in children under two months old, while JE is prevalent in children aged two months to 15 years old. Haemophilis influenza type B and Streptococcus pneumoniae are common in children aged two months to two years of age. JE is believed to be endemic in the whole country. JE vaccination is not yet included in the Department of Health-EPI programme.

Currently, AES is one of 26 diseases, syndromes and conditions included in the Philippines Integrated Disease Surveillance and Response (PIDS) coordinated by the National Epidemiology Center. JE data are captured through the AES surveillance. Other CNS infections
tracked by PIDSR are meningococcal disease, which is immediately notifiable, and bacterial meningitis, which is weekly notifiable. However, few cases are referred to laboratory for confirmation.

A series of EPI-laboratory-WHO meetings and consultations in 2010–2011 led to a country decision to strengthen AES surveillance as the Philippines considers introduction of JE vaccine. Pilot AES surveillance in five hospital sentinel sites under the National Epidemiology Center new vaccine surveillance was initiated. In 2013, further meetings between agencies led to a decision to pilot integrated AES-bacterial meningitis surveillance in two hospitals. After a JE outbreak in Tarlac province in 2003, Tohoku University (Japan) conducted sentinel surveillance in mosquitoes and swine from 2009 to 2011. Genotype 3 JE virus strain was detected from mosquitoes and from swine sera.

Laboratory quality assurance is in place, and the laboratory was accredited by WHO in 2013. Challenges for the laboratory system include case detection and investigation, full start of the strengthened AES surveillance, AES-bacterial meningitis pilot surveillance and slow feedback mechanism.

(7) Viet Nam (Ha Noi)

Ms Nguyen Thi Thu Thuy from NIHE in Ha Noi presented JE laboratory data from 2009 to 2012 and reported on the JE vaccination programme in Viet Nam. There are four JE surveillance sites in Viet Nam: one in the north, one in the central region and two in the south. The JE laboratory network in northern Viet Nam covers 28 provinces. Diagnostic methods used are serology using an in-house assay and Panbio IgM ELISA kit for JE and dengue, viral isolation using C6/36 cell line, RT-PCR and real-time PCR. In 2011, 169 JE suspected cases were reported; in 2012, the number grew to 196. Of these, 151 samples were collected in 2011 and 168 samples were collected in 2012. Among the samples tested, three (2%) were JE IgM positive in 2011 and nine (5.3%) were JE IgM positive in 2012. Viral Isolation was done on 94 CSF samples and no virus was isolated. In 2012, JE prevalence was higher in three provinces, namely, Thai Binh, Hai Pong and Quang Ngai. JE incidence is generally higher in the months of May to August. However, in 2011, three peaks were observed: a small peak from May to July, a higher peak from July to August, and another small peak from September to November. JE confirmed cases were found among children aged one year to 10 years old and youth over 15 years old. There were 29.7 million doses of JE vaccines distributed during 1998–2012. JE coverage during 1999–2012 was 93.9% for two doses and 94.8% for three doses.

The NIHE laboratory has participated in the WHO quality assurance programme since 2009. In 2012, the laboratory had a PT score of 90%. Specimens for confirmation were sent to NIID in Japan on a regular basis, and the most recent confirmatory testing concordance rate was 96.15%. A few challenges were mentioned such as: cases are missed, several cases without samples, revision and finalization of database for JE, stability of NIHE JE test kit and calibration of equipment.

(8) Viet Nam (Ho Chi Minh City)

Ms Huynh Phuong Thao from Pasteur Institute, Ho Chi Minh City presented on the JE vaccination system in Viet Nam and JE laboratory activities in Ho Chi Minh City. JE vaccination coverage is 94% for the first dose, 93% for the second dose and 96% for the third dose. The number of reported AES cases is decreasing, from 390 cases with 14 deaths in 2008 to 220 cases with four deaths in 2012. The national JE laboratory in Pasteur Institute is performing JE-Dengue IgM ELISA (Panbio and in-house kit), virus isolation and real-time RT-PCR. The 20 provincial
laboratories are using JE-Dengue IgM ELISA for the dengue national programme. Among AES cases reported in 2011 and 2012, 13% and 14% of cases, respectively, were JE positive and 5%–6% of cases were dengue positive. There is no clear seasonality of JE in southern Viet Nam. The laboratory quality assurance programme is well implemented. Pasteur Institute has been ISO 15189:2007 accredited since November 2010. The WHO PT score and confirmatory concordance rate were both 100% in 2012. A few challenges were identified, including training of staff in new techniques and biosafety, expansion of national JE surveillance system and fee for sample collection in provinces, and testing for other etiology of AES.

2.3.4 Reports from GSLs

(1) US CDC

Dr Barbara Johnson from US CDC reported on the activities of the Division of Vector-Borne Diseases (DVBD) to support the JE laboratory network. The Arbovirus Diseases Branch of DVBD, which serves as the WHO Collaborating Center for Reference and Research for Arboviruses has four functions: molecular virology, surveillance and epidemiology, entomology and ecology, and diagnostic and reference laboratory. Specimens are tested for all possible arboviruses from specific geographical regions, based on clinical information and volume of sample. Serological assays are performed on serum and CSF, while, viral RNA detection, nucleotide sequencing, virus isolation, immunofluorescence assays and dipstick are performed on mosquito pools, tissues, serum and CSF.

DVBD activities that support the JE laboratory network are (a) standardization of JE testing algorithm; (b) confirmatory testing of AES/AMES surveillance projects (more than 2000 CSF and serum tested by IgM ELISA and PRNT); (c) testing for other arboviruses (e.g. dengue, West Nile, Chikungunya); (d) JE laboratory training in the WHO South-East Asia and Western Pacific regions; (e) establishment of PT programme; (f) evaluation of commercial kits used in the JE laboratory network (Panbio, Inbios, Xcyton, NIV, Pune); and (g) development of JE reference serological panel to validate JE IgM assays. Three JE virus IgM ELISA kits with JE reference serological panel were evaluated; all three assays had sensitivities ≥92% and specificities ≥94%; cross-reactivity of dengue virus IgM positive samples in JE virus IgM ELISA is around 20%; and differential JE/dengue virus IgM detection assays have high specificity for dengue virus IgM, with no false-JE positive results. All assays used by the JE laboratory network have now been evaluated. Currently, JE reference panels prepared by US CDC for validation of ELISA assays and kits have good agreement between reference laboratories that evaluated the panel. Two in-house and two commercial JE IgM ELISA kits have been evaluated. US CDC/DVBD will continue to prepare, store, and distribute reference and proficiency panels, depending on specimen supply. However, more samples are needed to maintain reference panels. Other JE-related activities at DVBD relate to JE vaccine efficacy and include determination of immunogenecity of mouse brain-derived JE vaccine in archived specimens, duration of seroprotection following JE vaccine “booster” dose, duration of neutralizing antibody titres following single dose of Vero-cell derived vaccine (JE-VC; Ixiaro) in adults previously vaccinated with mouse-derived JE vaccine (Biken), and duration of neutralizing antibody titres following single JE-VC dose in adults previously vaccinated with JE-VC.

DVBD supports AES and JE surveillance through projects in Bangladesh and Cambodia, investigation of recurrent AES outbreaks in north-east India, and laboratory capacity-building at Eijkman Institute of Molecular Biology in Indonesia for arboviral detection and research. DVBD projects aimed at improving JE and other arbovirus diagnostics include validating use of filter paper to transport serological specimens, developing standardized ELISA format to facilitate differential diagnostic testing, and testing for “orphan” or newly emerging arboviruses (prototype
yellow fever), developing rapid tests for arboviruses (proof of concept JE IgM) and NS1 antigen detection rapid test (prototype yellow fever). Dr Johnson outlined the development in JE virus classification in the United States of America.

(2) Japan

Dr Tomohiko Takasaki from NIID reported on the development of JE vaccine and JE surveillance activities in Japan. Three JE cases were reported in 2008–2009, four cases in 2010 and nine cases in 2011 (one case imported from India) and two cases in 2012. Among the 21 cases confirmed since 2008, three were under seven years old, one was 10 years old, and the rest were adults (age range: 39 to 86 years old). In 2012, only two JE cases were reported in Japan because the density of Culex titaeniorhynchus was less than the previous summer. JE activity in pigs is also monitored in Japan, especially in prefectures suspected to be JE endemic.

The Vero cell-derived JE inactivated vaccine produced by Biken has been in the market since 2 June 2009. The vaccine produced by Kaketsuken has been licensed and has been in use since April 2011. Population immunity is being monitored annually, and data presented from 2006 showed that the percentage of the population that had detectable neutralizing antibodies to JE was high in the age group of two to 29 years old (range 50% to 90% of the population) compared to people over 30 years old (range 15% to 50% of the population).

NIID also functions as RRL and receives samples for confirmatory testing from Cambodia and the Lao People’s Democratic Republic.

Dr Takasaki discussed the application of the dengue non-structural protein 1 (NS1) ELISA for the confirmation of dengue virus infection and informed that it offered a new platform for diagnosis of dengue infection. It is also noted that dengue infection due to importation has increased in Japan over the years. Studies to test the efficacy of commercially available NS1 ELISA kits in the detection of the dengue virus NS1 antigen using a panel of serum samples, and comparison with efficacy of RT-PCR for detection of viral genome and antibody detection by ELISA for confirmation of dengue infection has been conducted by NIID. The results showed that NS1 antigen ELISA is a useful tool for confirming dengue virus infection in travellers, using serum samples in the acute phase or early convalescent phase, particularly when it is used in combination with RT-PCR and anti-dengue virus IgM ELISA. Dr Takasaki also discussed the development of an RNA stable tube for PCR testing and noted that dengue RNA is stable at room temperature for several months, and stable at 30 °C and at 40 °C for several weeks; hence, they are useful for PCR assays.

2.3.5 Report from GSL and RRL

(1) China

Dr Guo-dong Liang from China CDC presented on incidence and surveillance of JE in mainland China. He said that incidence of JE and deaths due to JE have decreased over the years from 9745 cases and 246 deaths in 2001 to 1763 cases and 59 deaths in 2012. A decrease has been noted predominantly since 2008, when JE vaccine was included into the routine EPI in China. In 2011, 1673 suspected JE cases were reported from 28 provinces. Xinjiang, Qinghai and Tibet are still JE-free.

During the JE surveillance meeting on 28 September 2011 in Chengdu, China CDC announced that China had set up a JE laboratory network. Ten provinces were included in the network (Guangdong, Yunnan, Sichuan, Shanghai, Zhejiang, Chongqing, Guizhou, Henan,
Shandong, Guangxi). With financial support from WHO, China CDC conducted two rounds of hands-on training on JE diagnosis, one in June 2011 and another in June 2012. As the RRL for provincial JE laboratories, China CDC is responsible for confirmatory testing and coordinates proficiency testing. A total of 873 samples (sera and CSF) from 740 cases were referred to the RRL by nine provincial JE laboratories in 2012 for confirmatory testing. The RRL distributed JE PT panels to 15 provincial CDCs in 2011 and to 10 JE laboratory network provincial laboratories in 2012. As the RRL, China CDC participates in WHO PT and has been accredited by WHO since 2011.

Entomological surveillance was also performed on 115,220 mosquitoes and 603 ticks from 10 provinces (Yunnan, Gansu, Hunan, Xinjiang, Jilin, Anhui, Hubei, Jiangxi, Guizhou, Fujian). Sixty-one virus strains were obtained including JE (n=18).

The first meeting of China’s JE laboratory network was held in Beijing on 10 October 2012. A total of 27 people participated in the meeting, including staff from the National Immunization Programme, China CDC Institute of Viral Disease Control and Prevention, and 10 provincial CDCs in the JE laboratory network. China is facing some challenges with its JE laboratory network, most notably, joining the WHO JE laboratory network. He said that until then, the RRL in China cannot share the results of confirmatory testing with WHO.

(2) Republic of Korea

Dr Myung Guk Han from the National Institute of Health, Korea CDC presented the activity of the laboratory and JE surveillance in the Republic of Korea. JE has been a notifiable disease since 1949 (5548 cases/2429 deaths). In 1968, JE vaccine was first imported into the Republic of Korea. The last JE epidemic was in 1982, and mandatory vaccination was introduced in 1983. From 1983 to 2009, there was an average of seven JE cases annually; however, the number of JE cases increased in 2010 and 2012. A dramatic shift in age distribution was noted in 2010, when the majority of JE cases were found among adults aged 30–59 years old. Diagnostic methods used are serological assays (ELISA and PRNT), molecular and virus isolation.

Korea CDC also functions as the RRL and receives samples for confirmatory testing from Malaysia and the Philippines.

JE virus detection was also performed in mosquito vectors. Culex mosquitoes (for JE virus) and Aedes mosquitoes (for other flaviviruses) were collected from May to October. The results of the SYBR Green-based real-time RT-PCR assay were published in the Journal of Virological Methods in 2010. The results revealed JE virus genotype 5 was detected in Gangwon province, two Chaoyang viruses were detected in Aedes vexans mosquitoes, and unclassified flaviviruses were detected in Aedes albopictus mosquitoes.

Some challenges of JE virus activity in the Republic of Korea still remain despite efforts to control the virus. JE virus genotype 5 has been detected since 2010, so the influence of the emergence of a new genotype should be investigated. There is no clear explanation of an abrupt increase in JE cases in 2010 and 2012. Since 2011, unclassified mosquito-borne flaviviruses have been detected throughout the country; thus, characteristics and pathogenicity should be studied through virus isolation.
2.3.6 PATH JE projects to support the Western Pacific Region

Dr Raj Shankar Ghosh from PATH gave a presentation on JE vaccine projects (2004–2012; 2012–2017) funded by Bill & Melinda Gates Foundation. The mission of these projects is to eliminate clinical JE and avoid the unnecessary death and disability caused by this disease in partnerships with national governments, WHO (Headquarters, South-East Asian and Western Pacific Regional Offices), US CDC (Arbovirus Diseases Branch), China National Biotech Group and in-country research organizations. Accomplishments of the JE project (2004–2012) include collection of data on JE disease burden from AES surveillance and data on vaccine safety, efficacy and cost-effectiveness for decision-making; negotiation of favourable public sector price for JE vaccine to enable large-scale vaccine adoption; and distribution of live attenuated SA 14-14-2 JE vaccine to more than 100 million children in Cambodia, India, Nepal, the Democratic People's Republic of Korea and Sri Lanka.

A five-year, multi-country JE project (2012–2017) is being supported in 13 countries: six in the South-East Asian Region (Bangladesh, Bhutan, Indonesia, Myanmar, the Democratic Republic of Korea and Timor-Leste), six countries in the Western Pacific Region (Cambodia, the Lao People's Democratic Republic, Papua New Guinea, the Philippines, Solomon Islands and Viet Nam) and one country in the Eastern Mediterranean Region (Pakistan). The goal of the project is to introduce JE vaccine in countries where AES surveillance is planned, and if data warrant introduction of vaccine. Proposed regional activities of the multi-country JE project are: (a) to identify regional training centres and to support the training of programme managers for JE vaccination planning and monitoring; (b) to coordinate biennial, biregional JE meetings for knowledge sharing between countries; and (c) to develop a web-based tool for in-country JE programme managers to access resource materials based on existing resources, such as Advanced Immunization Management (AIM) module. Activities of PATH in project countries include extending technical assistance to countries in planning and implementation of AES surveillance, and implementation of JE vaccination by adopting global strategies, practices and tools, and facilitating uninterrupted supply of JE vaccines.

3. CONCLUSIONS AND RECOMMENDATIONS

3.1 Polio Laboratory Network Session

A two-day meeting of the polio laboratory network in the Western Pacific Region was held from 11 to 12 March 2013 to review the progress of expanding real-time polymerase chain reaction (PCR) for intratypic differentiation (ITD) of polioviruses and vaccine-derived poliovirus (VDPV) screening among national laboratories and subnational laboratories in China, to identify the challenges and ways to strengthen the quality of the performances and to define/develop plans for expanding roles of the polio network laboratories to maintain polio-free status in the Region. Participants discussed the implementation of recommendations from the network's meeting held in September 2011 and the monitoring of laboratory performances including the outcomes of proficiency tests for virus isolation, poliovirus ITD and sequencing in 2011–2012. The network laboratories' experiences in the laboratory diagnosis of hand, foot and mouth disease (HFMD) and supplementary enterovirus and environmental surveillance were also presented.
3.1.1 Conclusions

The meeting concluded that the performance of the regional polio laboratory network has been sustained at polio-free-certification standard and that acute flaccid paralysis (AFP) surveillance activities have been efficiently supported. The network laboratories provided critical evidence in support of the continued polio-free status of the Region. As of March 2013, all 43 network laboratories are fully accredited including all seven polio laboratories with ITD function. All seven ITD laboratories passed proficiency testing (PT), and five ITD laboratories scored 100%. Five ITD laboratories also participated in polio sequencing PT, and four laboratories scored 100%. It is planned that seven laboratories will participate in polio sequencing PT in 2013.

The Region is making efforts to reduce the time to identify polioviruses by introducing real-time PCR for polio ITD and VDPV screening and the new virus isolation algorithm among subnational laboratories in China from 2013. From 2013, all network laboratories will use the new algorithm for virus isolation for testing AFP samples and WHO PT. After the introduction of real-time PCR for ITD in this Region in 2009, five laboratories introduced real-time PCR, while two laboratories (Hong Kong [China] and New Zealand) used conventional PCR for ITD until 2012. The second regional hands-on training on the real-time PCR for polio ITD and VDPV screening was held in December 2012 to expand real-time PCR to two ITD laboratories (Hong Kong [China] and New Zealand) and four national polio laboratories in the Philippines, the Republic of Korea and Viet Nam. The training introduced the new dual-stage real-time PCR method. After the training, the six laboratories began performing steps to gain proficiency in the techniques.

Polio network laboratories in the Region have been actively involved in supplementary enterovirus or environmental surveillance. In particular, the Chinese Center for Disease Control and Prevention (China CDC), which established a very extensive HFMD laboratory network based on existing polio and measles/rubella laboratories, provided support to Cambodia during the HFMD outbreak in 2012. The polio laboratories in Australia and Malaysia were involved in testing samples from environmental surveillance, and China polio network laboratories expanded the environmental surveillance to 11 provinces. Two wild poliovirus type 1 strains were detected from environmental samples collected in Hotan during the wild polio outbreak in Xinjiang. Sequencing of two wild poliovirus strains showed close homology to wild poliovirus strains detected from cases during the outbreak. The establishment of environmental and enterovirus surveillance in a number of countries in the Region has provided valuable data to support the polio-free status of the Region. During 2011–2012, VDPVs were detected from 17 individuals in China including one VDPV identified from a patient from Myanmar in 2012. Two VDPVs were also identified in southern Viet Nam in 2012.

During the wild polio outbreak in Xinjiang China in 2011, China’s polio laboratory network provided timely laboratory confirmation by introducing PCR detection in parallel to virus isolation. A total of 51 wild poliovirus type 1 strains were detected from AFP cases, contacts or healthy individuals during this outbreak. Through collaboration with the WHO global specialized laboratory in US CDC and the Pakistan National Institute of Health (NIH) laboratory, it was confirmed that wild poliovirus type 1 strains detected in China were closely related to viruses circulating in Pakistan. China’s polio laboratory network provided critical information to the national programmes until China was certified polio-free again during the 18th Regional Certification Committee (RCC) meeting in Beijing, China in November 2012.
Brief sessions on the new polio laboratory database, biorisk management, new vaccine laboratory networks and the hepatitis B control initiative in the Western Pacific Region were organized on the second day of the polio session.

3.1.2 Recommendations

(1) Full implementation of the new virus isolation algorithm in the Region including provincial laboratories in China

(a) With full implementation of new test algorithms in the Region, including in China, network laboratories should closely monitor the timeliness of testing and reporting: virus isolation results within 14 days and ITD results within seven days.

(b) Currently, China network laboratories report virus isolation within 18 days. Effort should be made to meet the global target of 14 days for reporting virus isolation results by the end of 2013. China CDC should closely monitor the implementation of the new algorithm among network laboratories to meet the 14-day turnaround time for virus isolation.

(2) Expansion of real-time PCR for ITD and VDPV screening

(a) Network laboratories (RITM Philippines, NIHE Viet Nam, Pasteur Institute Viet Nam and Korea CDC) that were trained on real-time PCR for polio ITD and VDPV screening in 2012 should complete four implementation steps including final PT as soon as possible and fully implement the technique by June 2013. During this period, the laboratories should refer all L20B positive isolates to designated regional reference laboratories (RRLs) within seven days.

(b) Once these laboratories successfully complete the implementation steps and get approval from the regional laboratory coordinator, they can perform polio ITD and VDPV screening and report results within seven days to the national programme and WHO. All non-Sabin-like strains should be referred for sequencing to designated sequencing laboratories within seven days.

(c) A new dual-stage real-time PCR method for ITD and VDPV screening developed by US CDC should be introduced among all ITD laboratories. These laboratories are encouraged to share the results with the US CDC polio team under Dr Dave Kilpatrick (US CDC), Dr Bruce Thorley (VIDRL) and the regional laboratory coordinator to successfully implement the dual-stage methods.

(d) All ITD laboratories including the current and new ITD laboratories are recommended to use the dual-stage real-time PCR for the testing of 2013 WHO ITD proficiency samples. The dual-stage real-time RT-PCR method is more sensitive than the standard method and does not require nucleic acid extraction of cell lysates. The method can also be directly applied to virus mixtures.

(e) Polio ITD and VDPV reagents: All ITD laboratories should use standardized kits/reagents provided by US CDC. The kits validated for this ITD procedure can be requested through WHO.
(f) Use of polio ITD laboratory checklist for annual WHO accreditation: Polio laboratories with ITD function that have successfully completed the implementation steps for establishing ITD testing after one year will be reviewed using the WHO ITD laboratory checklist after one year.

(3) Full implementation of real-time PCR for ITD and VDPV screening in China’s polio laboratory network

(a) China polio network laboratories which were trained on real-time PCR for ITD and VDPV screening in March 2012 and have passed the final PT can perform dual-stage real-time PCR for ITD and VDPV screening and report ITD results within seven days to China CDC by the third quarter of 2013. All non-Sabin-like strains and ITD discordant isolates should be referred to China CDC for sequencing within seven days.

(b) China CDC should ensure the quality of ITD testing in all provincial laboratories that introduced the methods including sequencing of all isolates.

(c) Provincial laboratories performing ITD testing will be assessed for ITD function during the on-site accreditation review starting from 2013.

(4) Polio laboratories without ITD capacity should refer L20B positive isolates to designated reference laboratories for characterization within seven days of detection.

(5) Timeliness of reporting wild poliovirus and VDPV detection: To reduce the risk of within-country and international spread of wild poliovirus or VDPV and the risk of outbreaks of poliomyelitis, all network laboratories should promptly report the detection of wild poliovirus or VDPV from any source including environmental samples to national authorities and WHO country and regional offices within 24 hours.

(6) Timeliness of reporting ITD results of polioviruses including non-AFP samples: All suspected polioviruses detected from any source should be rapidly confirmed using standardized ITD or sequencing method in WHO-accredited laboratories, and results should be reported to national authorities and WHO within seven days of detection.

(7) Sharing cell sensitivity testing results

(a) All network laboratories should continue to report results of cell sensitivity tests and titration experiments to the regional laboratory coordinator within 48 hours of test completion for review and for implementation of appropriate corrective actions early.

(b) The results should be presented in a graphic format by serotype for easy monitoring of trends over time.

(8) Sequencing PT and accreditation of polio sequencing laboratories

(a) ITD laboratories with proven sequencing capacity are encouraged to participate in polio sequencing PT and accreditation programme. Sequencing results should be reported within the required time frame.
(b) Primers for sequencing will be provided by US CDC in collaboration with WHO. Laboratories accredited for sequencing can provide nucleotide sequence data for programme use.

(9) 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 reference laboratories for further analysis. ITD laboratories with some problems in shipping isolates to sequencing laboratories can consider the use of FTA cards or RT-PCR products to ship non-infectious samples for further investigation.

(10) Data management and reporting

(a) The new standardized polio laboratory database can be introduced in network laboratories by the end of the second quarter in 2013 in order to meet all quality requirements. In addition, this will help to improve the timeliness for the data requirements in the framework of the polio endgame strategy.

(b) During the transition period, laboratory data including feed-forward files should be submitted to WHO and national EPI at least monthly, i.e. every 10th of the month in parallel with the current report format. Once the database is successfully established, the frequency of the reporting can be switched from monthly to weekly.

(c) For laboratories with their own database, it is recommended that the WHO Regional Office for the Western Pacific and network laboratories work together to interface the databases to accommodate necessary variables.

(d) Reporting non-AFP data: All polioviruses isolated from all sources, including those identified from non-AFP cases, environmental surveillance and other disease surveillance systems (e.g. enterovirus, respiratory viruses), can be reported to WHO using the new polio laboratory database.

(e) Reporting of polioviruses from patients aged 15 years and older: Polioviruses isolated from patients aged 15 years and older who present with clinical symptoms compatible with poliomyelitis or AFP should be reported using the same reporting format as for AFP data.

(11) 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.

3.1.3 Biorisk management

Laboratory safety is of utmost importance to the Global Poliomyelitis Laboratory Network (GPLN). This session provided an overview of biorisk management based on recent training materials developed by the WHO biosafety advisory group in order to strengthen laboratory capacity in this area.
Recommendations:

(1) It is noted that regional laboratory staff received training in biorisk management and the dissemination of this knowledge is important. Biorisk management training should be provided at the national and regional laboratory meetings and workshops to increase the knowledge and understanding of best practices by laboratory network staff.

(2) National polio laboratories should communicate with the ministry of health or other appropriate national biosafety authorities regularly to address relevant biosafety issues in the national polio laboratory, and each national polio laboratory will also be required to nominate a biosafety focal point who will liaise with WHO.

3.2 Measles and Rubella Laboratory Network Session

A two-day meeting of the measles and rubella laboratory network in the Western Pacific Region was held from 13 to 14 March 2013 to review the progress and identify the challenges facing measles and rubella laboratories. It was also necessary to present and discuss the Regional Committee’s resolution (WPR/RC63.R5) on measles elimination and acceleration of rubella control and the regional guidelines to verify measles elimination. The goal was to develop plans to further strengthen the molecular detection capacity and to ensure the performance of network laboratories to support measles elimination and rubella control goals in the Region.

3.2.1 Conclusions

The meeting concluded that measles and rubella network laboratories provided high-quality support to achieve the regional goal of measles elimination and rubella control by confirming suspected cases of measles and rubella and by identifying measles and rubella virus genotypes circulating in the Region.

The network consists of one global specialized laboratory (GSL) in Japan, three RRLs in Australia, China, and Hong Kong (China), 13 fully functional national measles and rubella laboratories, 31 provincial and 331 prefectural laboratories in China and three new subnational laboratories in Viet Nam and Malaysia. Among 48 laboratories for which WHO conducted on-site reviews for accreditation, 46 laboratories are fully accredited as of March 2013. Two laboratories with pending accreditation status need to improve laboratory performance by implementing recommendations from the previous accreditation visit. To ensure the quality of measles and rubella IgM testing in national laboratories, confirmatory testing has been conducted in WHO RRLs, and the two RRLs in Australia and Hong Kong (China) received a proportion of serum samples with retesting results provided to the national laboratories. The concordance rates of IgM testing for measles and rubella were >90% for most laboratories.

In 2011, 57 905 samples were tested for measles IgM by network laboratories, including 33 550 samples tested in China and 24 355 samples tested in non-China laboratories. Among samples tested, 26% were positive for measles (6017 from other countries and 8951 from China). A total of 16 586 samples were tested for rubella in non-China laboratories and 5332 samples (32%) were positive for rubella IgM. Approximately 5000 rubella-positive cases were also reported from China.

In 2012, 13 530 samples were tested for measles IgM among non-China laboratories, and more than 30 000 cases were tested for measles IgM in China. Among those samples tested, 2701
(20%) were measles positive among non-China laboratories, and 5922 cases were measles IgM positive in China. In 2012, 13 549 samples were tested for rubella IgM, and 1862 samples (13.7%) were positive for rubella IgM. Approximately 4300 cases were also reported from China.

An increased proportion of cases were laboratory-confirmed, and the proportion of cases with genotype information also increased in 2011 and 2012. While 39% of measles cases were laboratory-confirmed in 2010, 71.7% and 80.6% were laboratory-confirmed in 2011 and 2012, respectively. The proportion of measles cases with genotype information was 1.4% in 2010, 5% in 2011 and 8% in 2012 (including China). The proportion of rubella cases with genotype information was 1.9% in 2010, 6% in 2011 and 15% in 2012.

The timeliness of testing and reporting IgM results within seven days also improved from 48.1% in 2010 to 78.6% in 2011 and 96.1% in 2012. While four countries (Cambodia, Fiji Papua New Guinea and northern Viet Nam) did not achieve the 80% target of testing and reporting within seven days in 2011, only two countries (Lao People’s Democratic Republic and Papua New Guinea) could not achieve the 80% target in 2012.

Cambodia and Viet Nam made significant progress in achieving measles elimination by reporting zero cases and six cases in 2012, respectively. Malaysia continued to report a large number of measles cases (1582 cases in 2011 and 1829 cases in 2012). The Philippines reported 3829 measles cases in 2011 and 654 cases in 2012.

As the role of the measles and rubella laboratory network also extends to molecular surveillance, laboratories with virus isolation, molecular diagnostics and sequencing capabilities were encouraged to perform virus isolation, sequencing and genotyping. Genotype and sequence information for measles should be submitted to MeaNS, and rubella sequences can be submitted to RubeNS as soon as it is available. Genotype data on recent measles virus strains are available from all countries except the Pacific island countries.

In 2011-2012, the Hong Kong (China) RRL continued to provide excellent support to identify genotypes of measles and rubella viruses circulating in Cambodia, the Lao People's Democratic Republic, Macao (China), Malaysia, Mongolia, the Philippines and Viet Nam using confirmatory serum or virus isolation samples. Additional rubella genotyping was conducted for rubella-positive serum samples from Fiji, Papua New Guinea and other Pacific island countries.

Laboratories with the capacity to conduct virus isolation and molecular detection were further trained during the follow-up training in October 2012 in Hong Kong (China), and molecular practice panel samples for measles and rubella were distributed and tested by participating laboratories. Most laboratories obtained good scores for RT-PCR, genotyping and real-time PCR.

The completeness and timeliness of measles laboratory data reporting to the WHO Regional Office for the Western Pacific also improved to 77.5% and 61.8% in 2010, to 93% and 88% in 2011, and to 93% and 87% in 2012, respectively. China initiated reporting aggregate surveillance data including the number of laboratory-confirmed measles cases in 2011.

Genotyping data for measles and rubella from China and Hong Kong (China) were also shared with WHO Headquarters and the Regional Office for the Western Pacific on a monthly
basis. Hong Kong (China) also reported genotyping data for other national laboratories (Cambodia, Lao People's Democratic Republic and Philippines) in the Region to WHO.

Laboratory testing data for measles and rubella IgM and viral genotypes identified from each country have been included in the *Measles and Rubella Bulletin* of the WHO Regional Office for the Western Pacific since 2011.

Laboratory performance will be a critical component of verifying measles elimination in the countries. The recently published global framework for verification of measles and rubella elimination (*Weekly Epidemiology Review*, March 2013) describes five lines of evidence for determining whether a country or region has achieved measles and/or rubella elimination. Of these five lines of evidence, two are directly related to laboratory activities, namely, the presence of high-quality epidemiologic and laboratory surveillance systems and genotyping evidence that measles and rubella virus transmission is interrupted.

Within this framework, there are four core and one complementary performance indicators for WHO network laboratories:

**Core**

1. Proportion of measles network laboratories that are WHO-accredited for serological and, if relevant, for virological work (target: 100% of laboratories)

2. Proportion of serological results reported by the laboratory within four days of receiving the specimen (target: > 80%)

3. Proportion of laboratories (government and private) in the country that conduct measles diagnostic testing and that have effective quality assurance in place (target: 100%)

4. Proportion of virus detection and genotyping results (where appropriate) completed and reported within two months of receipt of specimen (target: ≥80% of specimens received)

**Complementary**

Completeness and timeliness of monthly reporting (including zero reporting) to the Regional Office for the Western Pacific of specimens received for serological and virological analysis (target: ≥80% of specimens received in laboratories)

**3.2.1 Recommendations**

1. **Timeliness of measles and rubella IgM testing and reporting:** All network laboratories should shorten the time spent on measles and rubella IgM testing and reporting from seven days to four days after the receipt of samples in the laboratory (target: 80% of samples) beginning in the second quarter of 2013 to be in line with the global framework for verifying elimination of measles and rubella and the draft guidelines on verification of measles elimination in the Western Pacific Region.

2. **Confirmatory testing**

   (a) The confirmatory testing mechanism of serum samples established in the Region should be maintained, and national laboratories should initiate sending a
representative 10% of samples or a minimum of 15 samples (for the laboratories which tested fewer than 150 samples per year) to the designated regional reference or global specialized laboratory, at least annually but preferably twice a year.

(b) A table including a line list of the samples and the raw data (OD readings) obtained by the national laboratory should be included and the shipment should be arranged by contacting the WHO Regional Office and the designated regional reference or global specialized laboratory.

(c) Before sending samples, national laboratories should consult the regional laboratory coordinator to confirm the number and selection of samples to be sent. Laboratories should consider sending samples for confirmation on filter paper to address shipping issues.

(d) Regional reference laboratories should consider testing IgM positive serum samples for the presence of viral RNA to expand the genetic databases for measles and rubella.

(3) Molecular surveillance: The network laboratories should continue making full efforts to obtain complete genotype and sequence information on measles and rubella viruses circulating in the Region using the proper molecular window and work in collaboration with the epidemiology group to differentiate imported cases from endemic cases.

(a) Laboratories should work with epidemiologists to obtain vital epidemiologic data, such as vaccination record, recent travel and/or close contact with measles/rubella cases.

(b) Virologic surveillance is a critical component of laboratory surveillance for measles and rubella. There is a critical need to expand the database of viral sequences from the Region especially for rubella. Laboratories are recommended to submit sequence information for measles viruses to MeaNS and for rubella viruses to RubeNS, as soon as it is available. Network laboratories including Australia, China, Hong Kong (China), Japan, Malaysia and Singapore, which currently share measles virus sequences through MeaNS, are encouraged to continue to share the sequences preferably on a “real-time” basis, or at least once a month.

(c) Laboratories that participated in the regional hands-on laboratory training workshops in 2010 and 2012 are encouraged to perform virus isolation and/or molecular detection of measles and rubella viruses including real-time RT-PCR and sequencing to identify the genotypes. Diagnostic RT-PCR (conventional or real-time) and genotyping RT-PCR kits are available from US CDC and can be requested through the WHO Regional Office for the Western Pacific. For laboratories that are initiating molecular testing, it is recommended to use the WHO protocols that were described in the training workshops.

(d) Network laboratories should work closely with surveillance groups or hospitals to receive virus isolation samples. The laboratory network can utilize the well-validated tools and samples available for enhancing molecular surveillance, where appropriate, such as oral fluid (Oracol kit), throat swab or urine samples for virus detection. Laboratories can consider using FTA cards for shipping clinical samples
for molecular testing, if necessary. Laboratories with difficulties maintaining cold-chain when shipping clinical samples for molecular testing should consider using FTA cards for shipment. However, samples shipped by FTA cannot be used for virus isolation. In addition, detection of rubella virus RNA and genotyping from clinical samples has not been studied to date.

(e) Laboratories are encouraged to communicate with national surveillance colleagues to inform them of the types and number of samples that are most appropriate for virus isolation. Ideally, samples should be collected simultaneously with the blood samples for serological diagnosis depending on local resources and facilities for transport and storage (refer to Annex 2).

(4) Quality assurance

(a) WHO IgM proficiency testing (PT)

Network laboratories including the Chinese provincial laboratories should include OD values when reporting the WHO global measles and rubella ELISA IgM PT. Validity criteria should be clearly indicated in the report.

All network laboratories should report PT results within 14 days from specimen arrival to VIDRL and the regional laboratory coordinator using the agreed reporting format. As the results can now be downloaded directly into the VIDRL database, participants are requested not to alter the reporting format.

(b) Quality assurance of prefectural laboratories

Provincial laboratories in China made impressive progress in ensuring the quality of prefectural laboratory performance by introducing annual PT, confirmatory testing and on-site visits. It is recommended that more support be provided from China CDC to provinces with limited human resources and laboratory capacity to obtain and maintain accreditation status and to introduce quality assurance programmes for prefectural laboratories.

(c) Molecular PT

WHO will introduce molecular PT with support of US CDC using a standard reporting form in 2013. Countries that participated in the molecular practice panels after the 2010 and 2012 hands-on trainings are encouraged to participate in the molecular PT annually. The results of molecular PT will be included in accreditation checklists for performing molecular testing.

(5) Use of dried blood spots (DBS)

(a) For countries using DBS samples as an alternative sampling method, adequate sampling to fill the circles should be ensured.

(b) For countries using DBS as an alternative sampling method, IgM results should be monitored closely for any discrepancy of results with serum samples. Any inadequate sample should be noted in laboratory testing records.

(6) Quality assurance of non-network laboratories and commercial laboratories
(a) In countries where most measles and rubella IgM testing is performed in non-network laboratories or private/commercial laboratories, it is important that the performance of those laboratories be comparable to network laboratories in timeliness and quality.

(b) The performance of non-network and commercial laboratories may be assessed through an external quality assessment programme, and pre-existing quality assurance data should be assessed by the national laboratories, where possible. The results of those assessments should be shared with WHO, where possible, and will be included in the national verification documentation.

(c) In countries with a low incidence of measles and rubella, national measles/rubella laboratories should consider confirming positive IgM results obtained in non-network laboratories.

(d) It is recommended that the ministry of health communicate with non-network laboratories involved in a significant proportion (eg. more than 20% of national samples) of measles and rubella testing.

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

(a) All network laboratories should store measles and rubella positive serum samples at -20 °C or lower for use as internal laboratory controls (in-house control samples) and for global PT panels. IgM positive serum samples collected within seven days after rash onset could be also used for virus identification/genotyping at designated WHO RRLs.

(b) Laboratories with stocks of measles/rubella IgM positive samples (preferably volumes larger than 0.5 ml) are requested to contact the regional laboratory coordinator to facilitate using these samples in the WHO PT and quality control programme.

(8) Testing algorithm of suspected measles, rubella and CRS cases

(a) All laboratories should develop an algorithm for testing samples from suspected cases of measles, rubella and CRS. This algorithm should be developed along with national epidemiologists and include the use of available laboratory methods for case classification. This algorithm should include a plan for referral of samples as necessary to RRLs and GSLs for tests that are not available at the national level (e.g. IgG avidity). (Guidelines for case classification in an elimination setting have been developed by the Pan American Health Organization and will be shared with the WHO Regional Office for the Western Pacific.)

(b) Network laboratories that do not perform measles testing of rubella IgM negative samples or rubella testing for measles IgM negative samples should devise a sampling plan to perform cross-check IgM testing. Given the importance of timely detection of measles cases, it is recommended that network laboratories perform measles and rubella IgM testing in parallel or initial measles IgM testing followed by rubella IgM testing unless there are ongoing rubella outbreaks.
(c) In a low incidence setting or when classification of an individual case is very important, alternative serological tests can aid in classification of suspect measles and rubella case(s). IgG testing, interpreted in the context of the known time course of IgM rise and IgG rise, can be helpful for case classification (e.g. rise in IgG for paired sera collected at optimum time points can confirm a case).

(d) Considering the rubella and CRS elimination initiative, network laboratories should be prepared to support laboratory surveillance for rubella and CRS and receive test samples from suspected rubella and CRS cases (refer to Annex 1).

(9) Use of measles and rubella IgG avidity tests: Fully validated measles and rubella avidity assays are readily available to support case classification. IgG avidity will be low for sera taken within three months of rash onset from an actual measles/rubella case. Commercial kits are available for rubella avidity and have been compared. Rubella avidity testing can be used to differentiate recent infection. Measles avidity testing can be used to differentiate recent and distant infection as well as to confirm vaccine failures. RRLs in the Region are encouraged to consider initiating avidity testing to provide an additional test to aid in case classification.

(10) Measles and rubella serosurveys: Documentation of population immunity will be an important component of the regional verification process. In some cases, serosurveys will be needed to assess vaccination coverage and population immunity. WHO will develop guidance for conducting serosurveys.

(11) Mumps testing: Laboratories with sufficient capacity should consider serological and/or molecular testing for mumps if this is consistent with national priorities.

(12) Data reporting

(a) Laboratory data should be reported to the WHO Regional Office on a monthly basis by the 10th day of each month. Reports should be sent to the regional laboratory coordinator and data management team (wpr_epidata@wpro.who.int).

(b) The revised MS Access data-reporting format is recommended to 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 that is mutually agreed upon.

(13) Communications and sharing data

(a) 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. Laboratories should also report delays in testing of samples and regular reporting of laboratory data to the WHO Regional Office. Laboratory and immunization/surveillance colleagues are encouraged to work together for harmonization of data and to ensure accurate and timely classification of suspected cases.
(b) Where feasible, it is recommended to use a unique case ID in laboratory records and databases, which is initially assigned by the epidemiological unit, and to request case IDs from the epidemiological unit if one is not documented on the laboratory request form or any other document accompanying the specimens.

(c) Network laboratories are requested to provide feedback on the laboratory data included in the *Measles and Rubella Bulletin*.

(14) Modification of WHO accreditation checklists: The measles laboratory network accreditation checklist will be modified to reflect the changes in the global framework for verifying elimination of measles and rubella (testing and reporting the IgM results within four days after the receipt of samples).

(15) Laboratories with pending accreditation status: Laboratories with pending accreditation status (Lao People's Democratic Republic and Xinjiang, China) should make further efforts to be fully accredited by WHO as soon as possible by implementing WHO recommendations from the previous on-site review.

Annex 1. Testing of samples from CRS surveillance

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

(ii) For suspected CRS cases, blood specimens should be collected at birth from the child and tested for rubella IgM. If the serum collected at birth is IgM negative, follow-up specimens need to be collected later and IgM testing repeated. If the second serum sample is again IgM negative, the case should be disqualified as CRS.

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

(iv) Samples from CRS patients are a source for virus isolation and genotyping. Almost all CRS infants excrete virus at birth and some continue to excrete virus for months after birth. After four months, the proportion of CRS infants shedding virus decreases to 50%–60%. For molecular testing, the optimal specimens from suspected CRS cases are nasopharyngeal secretions and urine. Surgically removed cataracts can also be a good source of virus.

(v) Because CRS patients can shed virus for up to 18 months in nasopharyngeal secretions and urine, molecular testing may be an option for classifying cases six months and older. Rubella diagnostic RT-PCR is a good choice for molecular testing of specimens taken from CRS patients.
Annex 2. Samples for virus isolation

(i) It is recommended to collect throat or nasopharyngeal swabs, nasal aspirates or urine samples as soon after rash onset as possible. Measles/rubella virus isolation is most successful when samples are collected on the first day of rash through five days following onset of rash.

(ii) The samples should be collected at the first contact with a suspected case of measles/rubella when the serum sample for IgM testing is drawn.

(iii) It is important to transport the samples to the laboratory with cold packs as soon as possible following sample collection since both measles and rubella viruses are sensitive to heat.

(iv) For urine samples, it is preferable to obtain the first urine passed in the morning. About 10–50 ml should be collected in a sterile container and held at 4 °C–8 °C before centrifugation and must not be frozen before the concentration process.

3.3 Japanese Encephalitis Laboratory Network Session

A one-day meeting of the Japanese encephalitis (JE) laboratory network in the Western Pacific Region was held on 15 March 2013 to review the progress of the recently established JE laboratory network in the Region, develop a plan to sustain the laboratory network activities and further strengthen the performance of network laboratories to support JE control in the Region.

3.3.1 Conclusions

The JE laboratory network was established to improve the diagnostic laboratory capability for JE case confirmation among countries either known or suspected to be endemic for JE in the Western Pacific Region, as recommended by the 17th TAG meeting in 2008. These countries include Cambodia, China, the Lao People's Democratic Republic, Malaysia, Papua New Guinea, the Philippines and Viet Nam. During 2008–2009, one GSL, two RRLs and seven national laboratories (Cambodia, the Lao People's Democratic Republic, Malaysia, Papua New Guinea, the Philippines, and northern and southern Viet Nam) were designated.

The second JE laboratory network meeting was held on 24 February 2010 as part of the second VPD laboratory network meeting. The third JE laboratory network meeting was held from 30 May to 1 June 2011 in conjunction with the Fifth Biregional Meeting on Japanese Encephalitis Prevention and Control in Vientiane, Lao People’s Democratic Republic.

To build regional laboratory capacity for JE testing, three regional hands-on training workshops were organized in 2009, 2010 and 2011. The Papua New Guinea laboratory was the last national laboratory to join the JE laboratory network among 10 laboratories in the Region.

For quality assurance of the JE laboratory network, the WHO annual proficiency tests for JE were successfully conducted from 2009 to 2012, and confirmatory testing has been conducted at least once a year to ensure the quality of JE laboratory testing among national laboratories in the region. WHO accreditation using the WHO JE laboratory checklist was initiated in 2010. Eight out of 10 laboratories have been accredited as of March 2013. The use of in-house controls was initiated in 2011.
3.3.2 Recommendations

Based on discussions and findings from network laboratories, the following recommendations were made.

(1) Detection of IgM in CSF or serum samples by ELISA is the most common diagnostic method for acute JE cases. Detection of genome or virus isolation from CSF samples is not recommended as a routine diagnostic method.

(2) Data management and reporting

(a) It is recommended that designated national, regional reference and global specialized laboratories continue to share their laboratory testing data with the WHO Regional Office for the Western Pacific by the 10th of every month using the WHO reporting form (both aggregate and case line list).

(b) For the Lao People’s Democratic Republic, it is encouraged that the Lao Oxford Mahosot Wellcome Trust Research Unit (LOMWRU) in Mahosot Hospital function as a subnational JE laboratory, sending monthly data to NCLE as well as the WHO country office and Regional Office for the Western Pacific.

(3) Confirmatory testing

(a) National laboratories are recommended to send a proportion of samples to designated RRLs and GSLs for confirmatory testing. It is recommended to send positive samples (excluding positive samples to be used for the preparation of in-house control samples) and equivocal samples and representative negative samples (in time and of geographical areas) for confirmation at least once a year.

(b) Aliquots of samples sent to RRLs and GSLs for confirmatory testing should be at least 100µl for CSF and 250µl for serum. Samples should be shipped undiluted in leak-proof, externally threaded, screw-cap vials on dry ice (CSF samples).

(c) RRLs and GSLs are required to complete confirmatory testing and provide feedback to national laboratories and the WHO Regional Office in the Western Pacific within 45 days of receiving the samples from the national laboratories.

(d) National JE laboratories, RRLs and GSLs are encouraged to explore/evaluate the use of filter paper for transporting/sending samples.

Timeline: Ongoing

(4) Proficiency testing

(a) It is recommended that all WHO JE network laboratories participate in the JE proficiency testing programme arranged by WHO. Testing of the proficiency panel should be completed and all results should be reported, including raw data, calculations, validity criteria, kit expiration date and results interpretation, using the designated reporting form within 14 calendar days of receipt of the proficiency panel. Timeliness of reporting will be reflected in PT scores; 5% will be deducted for every seven-day delay.
(b) National JE laboratories with enough volume of JE or dengue IgM-positive samples and negative CSF samples are requested to send those samples to the WHO GSL for future quality assurance purposes and for consideration for inclusion in the preparation of the annual JE proficiency panel. National JE laboratories may coordinate with the WHO Regional Office for the Western Pacific to contribute samples for PT.

(5) Quality assurance of subnational laboratories: In countries with subnational laboratories or sentinel sites where JE testing is conducted, the national laboratory should consider implementing quality assurance measures such as confirmatory testing, proficiency testing and training and share the results of those quality assurance measures with WHO.

(6) Communication

(a) It is recommended that the WHO JE network laboratories establish regular communication with their ministry of health, the national EPI and surveillance groups for JE control. Any missing epidemiological information should be collected by communicating with national surveillance colleagues.

(b) Laboratory data should be shared in a timely manner with the national EPI or surveillance colleagues to support the decision to target population groups for supplementary vaccination when introducing JE vaccine and to monitor vaccination impact after vaccine introduction.

(c) Countries with parallel surveillance systems for JE and AES are encouraged to collect and harmonize available laboratory data from different sources as much as possible.

(d) Integration of JE surveillance with other types of surveillance (invasive bacterial - VPD, national notifiable diseases, etc.) should be considered where feasible to increase efficiency and reduce resource requirements.

(7) Training and meetings

(a) To address staff turnover, ensure/improve the quality of laboratory performance and sustain capacity for JE laboratory testing, hands-on training courses may be held as needed. The next WHO hands-on training is due in September 2013. Inter-regional workshops could be considered.

(b) Laboratory staff members from designated national or subnational JE laboratories who are directly involved in the testing of JE should participate in the training.

(c) The next JE laboratory network meeting could be jointly held with the biregional JE meeting.
# PROVISIONAL TIMETABLE

<table>
<thead>
<tr>
<th>Time</th>
<th>Monday, 11 March</th>
<th>Time</th>
<th>Tuesday, 12 March</th>
<th>Time</th>
<th>Wednesday, 13 March</th>
<th>Time</th>
<th>Thursday, 14 March</th>
<th>Time</th>
<th>Friday, 15 March</th>
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</thead>
<tbody>
<tr>
<td>08:00–08:30</td>
<td>Registration</td>
<td>08:30–09:00</td>
<td>7. Laboratory quality assurance</td>
<td>08:00–08:45</td>
<td>Session II: Measles and Rubella Laboratory Network</td>
<td>08:00–08:30</td>
<td>25. Measles/rubella serosurvey</td>
<td>08:00–08:30</td>
<td>Session III: Japanese Encephalitis Laboratory Network</td>
</tr>
<tr>
<td>08:30–09:00</td>
<td>1. Opening session</td>
<td>08:00–08:30</td>
<td>(a) Report on proficiency testing: Virus isolation</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(a) Standardization of measles serosurvey</td>
<td>08:00–08:30</td>
<td>Registration</td>
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<tr>
<td></td>
<td>(a) Opening remarks</td>
<td>08:00–08:30</td>
<td>(b) Reporting on intratypic differentiation (ITD) testing</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(b) Experience in China laboratory</td>
<td>08:00–08:30</td>
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<tr>
<td></td>
<td>(b) Self introduction</td>
<td>08:00–08:30</td>
<td>(c) Laboratory quality assurance of China polio laboratory network: introduction of new algorithm</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(c) Experience in Japan laboratory</td>
<td>08:00–08:30</td>
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<tr>
<td></td>
<td>(c) Administrative announcements</td>
<td>08:00–08:30</td>
<td>(d) Monitoring of cell sensitivity testing</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
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<td>08:30–09:00</td>
<td>33. Opening session</td>
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<tr>
<td></td>
<td>(d) Election of chairperson and rapporteur</td>
<td>08:00–08:30</td>
<td>(e) Accreditation of an ITD and sequencing laboratories</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(a) Self introduction</td>
<td>08:30–09:00</td>
<td>(a) Self introduction</td>
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<tr>
<td>09:00–10:30</td>
<td>2. Polio Endgame Strategy and Regional Updates on maintaining polio-free status in the Western Pacific Region</td>
<td>08:00–08:30</td>
<td>16. Opening session</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(b) Administrative announcements</td>
<td>08:30–09:00</td>
<td>(b) Administrative announcements</td>
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<tr>
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<td>(a) Polio endgame strategy and WPPO update on the polio eradication initiative and next steps</td>
<td>08:00–08:30</td>
<td>(a) Self introduction</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td></td>
<td>09:00–09:40</td>
<td>34. Overview of Japanese encephalitis (JE) control in the Western Pacific Region</td>
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<tr>
<td></td>
<td>(b) Global WPV transmission and status of global poliovirus laboratory network</td>
<td>08:00–08:30</td>
<td>(b) Administrative announcements</td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(a) JE/AES surveillance and JE vaccination in the Region</td>
<td>09:00–09:40</td>
<td>(a) JE/AES surveillance and JE vaccination in the Region</td>
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<td></td>
<td>(c) Regional updates of poliovirus laboratory network – expansion of ITD labs</td>
<td>08:00–08:30</td>
<td></td>
<td>09:00–09:10</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(b) Progress of Western Pacific region JE Laboratory Network</td>
<td>09:00–09:40</td>
<td>(b) Progress of Western Pacific region JE Laboratory Network</td>
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<tr>
<td>10:00–11:00</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>17. Overview of global and regional measles elimination and rubella control initiatives</td>
<td>10:20–10:30</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(a) Improving global rubella virus surveillance</td>
<td>09:40–10:20</td>
<td>35. Quality Assurance for JE laboratory</td>
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<tr>
<td>10:30–11:00</td>
<td>COFFEE BREAK</td>
<td>08:00–08:30</td>
<td>(a) Global and regional updates on eliminating measles and rubella</td>
<td>10:20–10:30</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(b) Rubella and congenital rubella syndrome surveillance in China</td>
<td>09:40–10:20</td>
<td>(a) Analysis of results of the 2011-2012 JE proficiency test</td>
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<td>11:00–11:40</td>
<td>3. Detection of Vaccine-derived poliovirus (VDPV) and VDPV Meeting and Expansion of ITD labs</td>
<td>08:00–08:30</td>
<td>(b) Progress of global measles and rubella laboratory network</td>
<td>10:20–10:30</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(c) Rubella and CRS surveillance in Japan</td>
<td>09:40–10:20</td>
<td>(b) Confirmatory testing and accreditation of JE laboratories in WPR</td>
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<td></td>
<td>(a) Global updates on VDPV detection and outcomes of the 2012 VDPV meeting</td>
<td>08:00–08:30</td>
<td>(c) Updates - regional measles and rubella laboratory networks</td>
<td>10:20–10:30</td>
<td>Discussion</td>
<td>08:00–08:30</td>
<td>(d) Rubella molecular surveillance in U.K.</td>
<td>09:40–10:20</td>
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<td>(b) VDPV surveillance during 2011-2013 in China</td>
<td>08:00–08:30</td>
<td></td>
<td>10:20–10:35</td>
<td>Discussion</td>
<td>08:00–08:30</td>
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<td>10:20–10:35</td>
<td>Discussion</td>
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<td>11:40–12:00</td>
<td>Discussion</td>
<td>08:00–08:30</td>
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<td>08:00–08:30</td>
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<td>11:00–12:00</td>
<td>9. Detection of poliovirus from non-AFP specimens and Environmental Surveillance</td>
<td>10:30–11:30</td>
<td>18. Quality assurance</td>
<td>11:00–12:00</td>
<td>Discussion</td>
<td>11:00–12:05</td>
<td>36. Country Reports – Part I</td>
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<td>(a) Environmental surveillance of poliovirus and non-polio enteroviruses in China</td>
<td>10:30–11:30</td>
<td>(a) Measles IgM proficiency test updates and confirmatory testing</td>
<td>11:00–12:00</td>
<td>Discussion</td>
<td>11:00–12:05</td>
<td>(a) Cambodia</td>
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<tr>
<td></td>
<td>(b) Australia experience</td>
<td>10:30–11:30</td>
<td>(b) Confirmatory testing in Hong Kong (China)</td>
<td>11:00–12:00</td>
<td>Discussion</td>
<td>11:00–12:05</td>
<td>(b) Fiji</td>
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<tr>
<td></td>
<td>(c) Malaysia experience</td>
<td>10:30–11:30</td>
<td>(c) China Laboratory Network: confirmatory testing and PT</td>
<td>11:00–12:00</td>
<td>Discussion</td>
<td>11:00–12:05</td>
<td>(c) Lao People's Democratic Republic</td>
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<tr>
<td>12:00–12:15</td>
<td>Discussion</td>
<td>10:30–11:30</td>
<td>(d) Quality assurance programme for molecular techniques</td>
<td>11:00–12:00</td>
<td>Discussion</td>
<td>11:00–12:05</td>
<td>(d) Mahasot hospital</td>
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<tr>
<td>11:30–11:45</td>
<td>Discussion</td>
<td>10:30–11:30</td>
<td>27. Reports from global specialized laboratories (GLS) and regional reference laboratories (RRL)</td>
<td>11:00–12:00</td>
<td>Discussion</td>
<td>12:05–12:20</td>
<td>Discussion</td>
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# PROVISIONAL TIMETABLE

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>12:00–13:00</td>
<td><strong>LUNCH BREAK</strong></td>
</tr>
<tr>
<td>13:00–14:00</td>
<td><strong>Country Reports and Experience</strong></td>
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<tr>
<td>13:15–14:00</td>
<td>Experience with real-time PCR and sequencing of AFP and Non-AFP samples</td>
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<tr>
<td>13:15–14:00</td>
<td>(a) Japan</td>
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<td>13:15–14:00</td>
<td>(b) Australia</td>
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<tr>
<td>13:15–14:00</td>
<td>(c) China: Report on introduction of new virus isolation algorithm and ITD expansion among provincial laboratories</td>
</tr>
<tr>
<td>13:15–14:00</td>
<td>(a) Hand, foot and mouth disease surveillance in China</td>
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<tr>
<td>13:15–14:00</td>
<td>(b) Korea experience</td>
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<tr>
<td>13:15–14:00</td>
<td>(c) Japan experience</td>
</tr>
<tr>
<td>13:15–14:00</td>
<td><strong>Experience of polio laboratory network for the lab diagnosis of hand, foot and mouth disease and other enteroviruses</strong></td>
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<tr>
<td>14:00–14:15</td>
<td><strong>Discussion</strong></td>
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<tr>
<td>14:15–15:00</td>
<td><strong>Country reports – Part I</strong></td>
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<tr>
<td>15:00–15:15</td>
<td>11. New polio lab database</td>
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<tr>
<td>15:15–15:30</td>
<td><strong>Discussion</strong></td>
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<tr>
<td>15:30–16:00</td>
<td><strong>COFFEE BREAK</strong></td>
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<tr>
<td>16:00–17:15</td>
<td><strong>Country reports of national polio laboratories – Part II</strong></td>
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<tr>
<td>16:30–16:45</td>
<td>22. Country reports – Part II</td>
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<tr>
<td>16:00–16:45</td>
<td>(a) Malaysia</td>
</tr>
<tr>
<td>17:00–17:20</td>
<td>23. Use of Alternative sampling</td>
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<tr>
<td>17:00–17:30</td>
<td>(a) Use of serum samples for genotyping, Hong Kong</td>
</tr>
<tr>
<td>17:30–18:30</td>
<td>32. Conclusions and recommendations of session II</td>
</tr>
<tr>
<td>18:00–18:45</td>
<td><strong>Closing session</strong></td>
</tr>
<tr>
<td>18:00</td>
<td><strong>RD’S RECEPTION</strong></td>
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</table>

**Note:**
- **Part I** includes Experience of polio laboratory network for the lab diagnosis of hand, foot and mouth disease and other enteroviruses, with a focus on countries like the Republic of Korea, New Zealand, Malaysia, Hong Kong, and China.
- **Part II** includes discussions on country reports, with emphasis on the experience of countries such as Cambodia, Fiji, Lao People’s Democratic Republic, and Papua New Guinea.
- **Part III** covers additional topics related to vaccines and laboratory networks, with sessions on proposals and recommendations.
LIST OF PARTICIPANTS, TEMPORARY ADVISERS, OBSERVERS, AND SECRETARIAT

POLIOMYELITIS SESSION, 11–12 March 2013

1. PARTICIPANTS

AUSTRALIA

Dr Bruce Thorley, Senior Medical Scientist, Head, WHO Regional Poliomyelitis Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn Street, North Melbourne 3051.
Tel no.: 61 3 9342 2607. Fax no.: 61 3 9342 2665.
E-mail: bruce.thorley@mh.org.au

CHINA

Dr Xu Wenbo, Chief of National Laboratory for Measles, Institute of Viral Disease Control and Prevention China Center for Disease Control and Prevention, 155# Changbai Road, ChangPing District, Beijing 102206.
Tel no.: 8610 5890 0187. Fax no.: 8610 5890 0187.
E-mail: wenbo_xu1@yahoo.com.cn

Dr Zhang Yong, Deputy Chief, National Laboratory for Polio, Institute of Viral Disease Control and Prevention, China Center for Disease Control and Prevention, 155# Changbai Road, ChangPing District, Beijing 102206.
Tel no.: 8610 5890 0183. Fax no.: 8610 5890 0184.
E-mail: yongzhang75@sina.com

Dr Yu Wenzhou, Professor, National Immunization Program, Chinese Center for Disease Control and Prevention, 27 Nanwei Road, Beijing.
Tel no.: 8610 630279466. Fax no.: 8610 630279468.
E-mail: wenzhouyu69@hotmail.com

Dr Tan Xiaojuan, Assistant Researcher, National Institute of Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155# Changbai Road, Changping District, Beijing102206.
Tel no.: 8610 58900185. E-mail: tan.xiao.juan@hotmail.com

HONG KONG (CHINA)

Dr Yee Chi Lo, Janice, Consultant Medical Microbiologist, Public Health Laboratory Centre, Department of Health, Hong Kong SAR
9/F 382 Nam Cheong Street, Shek Kip Mei, Kowloon
Tel no.: 852 2319 8254. Fax no.: 852 2776 5758.
E-mail: janicelo@dh.gov.hk

JAPAN

Dr Hiroyuki Shimizu, Chief, Laboratory of Enteroviruses, Department of Virology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama-shi, Tokyo 208-0011
Tel no.: 81 42 561 0771. Fax no.: 81 42 561 4729.
E-mail: hshimizu@nih.go.jp
MALAYSIA
Mr Mohd Apandi bin Yusof, Research Officer, Virology Unit, Infectious Disease Research Centre, Institute for Medical Research, Jalan Pahang 50588 Kuala Lumpur. Tel no.: 603 2616 2671. Fax no.: 603 2693 8094 Mobile no.: 013 8453167. E-mail : apandi@imr.gov.my

MONGOLIA
Dr Purev Suvd, Head of National Polio Laboratory, National Center for Public Health, Enkhtaivan Avenue 17, Baynzurkh District, Ulaanbaatar 13381. Tel no.: 976 99 816817. Fax no.: 976 11 45266. E-mail : suvd02@yahoo.com
Dr Sambuu Anand, Surveillance Officer, EPI, Department of Immunization, National Center of Communicable Diseases, Baynzurkh District, Nam Yan Ju Street, Ulaanbaatar. Tel no.: 976 9925 8021 E-mail : anand_sambuu@yahoo.com

NEW ZEALAND
Dr Sue Huang, Senior Science Leader-Virology, Communicable Disease Group, Institute of Environmental Science and Research, 66 Ward Street, Wallaceville, Upper Hutt. Tel no.: 64 4 529 0606. Fax no.: 64 4 529 0601. E-mail : sue.huang@esr.cri.nz

PHILIPPINES
Dr Amado O. Tandoc III, Medical Specialist III, Head, Virology Department, Research Institute for Tropical Medicine, 9002 Research Drive, FCC Compound, Alabang, Muntinlupa City 1781. Tel no.: 632 8098120. Fax no.: 632 8097120. E-mail : amado.tandocmd@gmail.com
Dr Maria Joyce Ducusin, Medical Specialist IV, National Center for Disease Prevention and Control, Building 14 San Lazaro Compound, Sta. Cruz, Manila. Fax no.: 632 7329956. Mobile no.: 63 9175725992. E-mail : juducusin@yahoo.com

REPUBLIC OF
Dr Doo Sung Cheon, Deputy Scientific Officer, Division of Vaccine Research, Center for Infectious Disease, National Institute of Health, Korea Centers for Disease Control and Prevention, OHTAC, 187 Osongsaemyeong2-ro, Osong-eup, Cheongwon-gun, Chungbuk 363-651. Tel no.: 82 43 719 8151. Fax no.: 82 43 719 8189. E-mail : cheonds@hanmail.net; cheonds@daum.net

SINGAPORE
Dr Lui Sook Yin, Scientific Officer, Singapore General Hospital Department of Pathology, #01-01B Outram Road, Singapore 169608. Tel. No.: 65 63265435. Fax No.: 65 63234972. Mobile No.: 65 91779985. E-mail : lui.sook.yin@sgh.com

VIET NAM
Dr Nguyen Thi Hien Thanh, Vice Head, Virology Department Head, Enterovirus Laboratory, Manager of Measles and JE Laboratories National Institute of Hygiene and Epidemiology, No. 1, Yersin Street, Hanoi 10000. Tel no.: 04 3 9726851 ext 218. Fax no.: 04 3 9726850 E-mail : thanhpoliolab@yahoo.com; thanh.enterovirus@nihe.org.vn
VIET NAM

Dr Nguyen Thi Thanh Thao, Head, Laboratory Enteric Viruses, Pasteur Institute 167 Pasteur Street, District 03, Ho Chi Minh City
Tel no.: 84 8 38 202 878. Fax no.: 84 8 38 231 419.
E-mail : tthao1103@yahoo.com

Dr Phi Van Kien, Officer, Communicable Disease Control Division
Ministry of Health, 135 Nui Truc lane, Ba Dinh District, Hanoi.
Tel no.: 84 4 38456255. Mobile no.: 84 986923186.
E-mail : phivankien@gmail.com

Dr Dang Thi Thanh Huyen, Team Leader of VPDs Surveillance and Statistics Unit, National Expanded Programme on Immunization
National Institute of Hygiene and Epidemiology, No. 1, Yersin Street, Hanoi 10000. Tel no.: 043 9721 334. Fax no.: 043 8213 782
E-mail : epi.huyen@gmail.com

2. TEMPORARY ADVISERS

Dr Olen Martin Kew, Associate Director, Global Laboratory Science, National Center for Immunization and Respiratory Diseases, Division of Viral Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop C-22, Atlanta, Georgia 30333, United States of America.
Tel no.: 404 639 3940. Fax no. 404 639 4011. E-mail : omk1@cdc.gov

Dr Paul Rota, Chief, Measles Virus Section, Centers for Disease Control and Prevention, Mailstop C-22, 1600 Clifton Road, Atlanta, Georgia 30033, United States of America.
Tel no.: 404 639 3512. Fax no.: 404 639 4178. E-mail : par1@cdc.gov

Dr Min-hsin Chen, Associate Service Fellow, Rubella Virus Section, United States Centers for Disease Control and Prevention, Mailstop C-22, 1600 Clifton Road, Atlanta, Georgia 30033, United States of America, Tel no.: 404 639 3508. E-mail : zvp8@cdc.gov

Dr Barbara Johnson, Diagnostic and Reference Laboratory, Arbovirus Diseases Branch Division of Vector-Borne Infectious Diseases (DVBID), Centers for Disease Control and Prevention (CDC), 3150 Rampart Road, Building 401, Room 3-322, Fort Collins, Colorado 80521, United States of America. Tel no.: 1970 266 3543. Fax no.: 1970 221 6441.
E-mail : bfj9@cdc.gov

Dr Michael Catton, Director, Victorian Infectious Diseases Reference Laboratory, Locked Bag 815, Carlton South, Victoria 3053, Australia. Tel no.: 61 3 9342 2636. Fax no.: 61 3 9342 2666.
E-mail : mike.catton@mh.org.au

3. OBSERVERS

Dr Pei Yun Shu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2278 3992
Annex 2

Dr Ho-Sheng Wu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2783 7779

Mr Ricardo A. Oraya, Jr., Surveillance Officer for VPDs, National Epidemiology Center Department of Health, Building 19, 2nd Floor, San Lazaro Compound, Sta. Cruz, 1003, Manila Tel no.: 63 2 651 7800 local 2930. Mobile no.: 63 916 2643170. E-mail : ricardo.oraya@yahoo.com

Dr Ji-Yeon Hyeon, Staff Scientist, Division of Vaccine Research, Center for Infectious Diseases National Institute of Health, Korea Centers for Disease Control and Prevention, 187 Osong, Chung-buk, 363-651, Republic of Korea. Tel no.: 82 43 719 8152. E-mail : sh702@konkuk.ac.kr

Dr Chikako Kataoka, Department of Virology II, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan. E-mail : ckataoka@nih.go.jp

Dr Lea Necitas Apostol, National Polio Laboratory Technical Supervisor, Research Institute for Tropical Medicine, Filinvest Corporation City Compound, Alabang, Muntinlupa City 1781 Philippines. Tel no.: 632 8072628. E-mail : leinetcitas9780@yahoo.com

Ms Leonibel A. Reyes, National Polio Laboratory Technical Staff, Research Institute for Tropical Medicine, Filinvest Corporation City Compound, Alabang, Muntinlupa City 1781, Philippines. Tel no.: 632 8072628. E-mail : leonibel_agajona@yahoo.com

MEASLES SESSION, 13–14 March 2013

1. PARTICIPANTS

AUSTRALIA Ms Vicki Vasiliki Stambos, Medical Scientist, WHO Measles Regional Reference Laboratory, Victorian Infectious Diseases Reference Laboratory 10 Wreckyn Street, North Melbourne, Victoria 3051. Tel no.: 61 3 9342 3920. Fax no.: 61 3 9342 3930. E-mail : vicki.stambos@mh.org.au

Mr Matthew Brian Kaye, Medical Scientist, Virology Laboratory, Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn Street, North Melbourne, Victoria 3051. Tel no.: 61 3 9342 2628. Fax no.: 61 3 9342 2629. E-mail : matthew.kaye@mh.org.au

BRUNEI Osmali Osman, Chief Scientific Officer Ministry of Health DARUSSALAM Bandar Seri Begawan BB3910. Tel no.: 6732221821 ext. 102. Fax no.: 6738971080. E-mail : bazil17112003@yahoo.com

CAMBODIA Mr Buth Sokhal, Deputy Director, National Institute of Public Health, Lot #2, Kim Yi Sung Boulevard, Sangkat Boeng Kok II, Khan Tuol Kork Phnom Penh. Tel no.: 855 16 888 889. Fax no.: 855 23 882 889. E-mail: buthsokhal@yahoo.com
CAMBODIA
Mr Am Chanthan, Head of Immunology Unit (Laboratory),
National Institute of Public Health, Lot #2, Kim Yi Sung Boulevard
Sangkat Boeng Kok II, Khan Tuol Kork, Phnom Penh.
Tel no.: 855 12 881 196. Fax no.: 855 23 882 889.
E-mail : am.chanthan07@gmail.com

Mrs Ngeth Savry, Immunization Officer, National Immunization Programme
Ministry of Health, No. 6A Prek Leap Khan Russey Keo, Phnom Penh
Tel no.: 855 12 679 896. Fax no.: 855 23 426 257.
E-mail : ngethsavry@yahoo.com

CHINA
Dr Xu Wenbo, Chief of National Laboratory for Measles
Institute of Viral Disease Control and Prevention, China Center for Disease
Control and Prevention, 155# Changbai Road, ChangPing District,
Beijing 102206. Tel no.: 8610 5890 0187. Fax no.: 8610 5890 0187.
E-mail : wenbo_xu1@yahoo.com.cn

Dr Mao Naiying, Associate Researcher, National Institute of Viral Disease
Control and Prevention, Chinese Center for Disease Control and Prevention
155# Changbai Road, ChangPing District, Beijing 102206.
Tel no.: 8610 5890 0188. Fax no.: 8610 5890 0188.
E-mail : maonaiying@hotmail.com

Dr Zhang Yan, Associate Researcher, National Measles Laboratory
National Institute of Viral Disease Control and Prevention
155# Changbai Road, ChangPing District, Beijing 102206.
Tel no.: 8610 5890 0188. Fax no.: 8610 5890 0188.
E-mail : zhangyanft@hotmail.com

Dr Xiong Ying, Director, Disease Testing Department
Jiangxi Provincial Center for Disease Control and Prevention
555 East Beijing Road, Nanchang, Jiangxi 330029.
Tel no.: 679 188319813. Fax no.: 8679 188319813.
E-mail : xiongying8300087@126.com

Dr Zhang Hong, Director, Micro-organism Examination Department
Hunan Provincial Center for Disease Control and Prevention
450 Furong Middle Road sec. 1, Changsa 410005, Hunan.
Tel no.: 86 731 84305928. E-mail : hnzhangh67@yahoo.com.cn

Dr Yu Wenzhou, Professor, National Immunization Program
Chinese Center for Disease Control and Prevention, 27 Nanwei Road,
Beijing. Tel no.: 8610 630279466. Fax no.: 8610 630279468.
E-mail : wenzhouyu69@hotmail.com

Dr Cui Aili, Associate Researcher, National Measles Laboratory
National Institute of Viral Disease Control and Prevention
Chinese Center for Disease Control and Prevention, 155# Changbai Road,
Changping District, Beijing. Tel no.: 8610 58900188.
Fax no.: 8610 58900188. E-mail : cuiaili@yahoo.com
Annex 2

**CHINA**
Dr Tan Xiaojuan, Assistant Researcher, National Institute of Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention
155# Changbai Road, Changping District, Beijing
Tel no.: 8610 58900185. E-mail: tan.xiao.juan@hotmail.com

**FIJI**
Dr Talica Vakacolata, Senior Laboratory Technician
Fiji Centre for Communicable Disease Control, Mataika House, Building 30
Tanavua Hospital Complex, G.P.O. Box 16346, Suva. Tel no.: 7070768
E-mail: tcabe25@gmail.com

Dr Prem Sanjeev Singh, Acting Senior Medical Officer,
Fiji Centre for Communicable Disease Control, Mataika House, Building 30
Tanavua Hospital Complex, G.P.O. Box 16346, Suva. Tel no.: 3320066
Fax no.: 332034. E-mail: premdrsingh@gmail.com

**HONG KONG**
Dr Janice Lo, Consultant Medical Microbiologist, Public Health Laboratory Centre, Department of Health, HK SAR, 9/F 382 Nam Cheong Street, Shek Kip Mei, Kowloon. Tel no.: 852 2319 8254.
Fax no.: 852 2776 5758. E-mail: janicelo@dh.gov.hk

Dr Jasmine Kwong, Medical and Health Officer, Public Health Laboratory Centre, Department of Health, HK SAR, 9/F 382 Nam Cheong Street, Shek Kip Mei, Kowloon. Tel no.: 852 2319 8357. Fax no.: 852 2319 5989
E-mail: mo_phls9@dh.gov.hk

**JAPAN**
Dr Katsuhiro Komase, Head of Laboratory of Measles Virus, Department of Virology III, National Institute of Infectious Diseases, 4-7-1 Gakuen,
Musashimurayama-shi, Tokyo 208-0011. Tel no.: 81 42 561 0771 ext.3707; 81 42 848 7061 (direct). Fax no.: 81 42 561 1960.
E-mail: kkomase@nih.go.jp

**LAO PEOPLE'S DEMOCRATIC REPUBLIC**
Dr Phengta Vongprachanh, Director, National Center for Laboratory and Epidemiology, Ministry of Health, Km3 Thadeua Road, Sisattanak District, Vientiane. Tel no.: 856 21 315858. Fax no.: 856 21 315858.
E-mail: v.phengta@gmail.com

Mr Virasack Som Oulay, Staff of Sero-Virology Laboratory, National Center for Laboratory and Epidemiology, Ministry of Health, Km3 Thadeua Road, Sisattanak District, Vientiane. Tel no.: 856 21 312858.
Fax no.: 856 21 312351; 856 21 315347.
E-mail: v.phengta@gmail.com

Dr Chansay Pathammavong, Deputy Manager of National Immunization Program, Mother and Child Health Centre, Ministry of Health, Km3 Thadeua Road, Sisattanak District, Vientiane,
Tel no.: 856 021 312352. Fax no.: 856 021 312120.
E-mail: chansay_epi@yahoo.com
Annex 2

MALAYSIA
Madam Norizah binti Ismail, Science Officer, National Public Health Laboratory, Ministry of Health Malaysia, Lot 1853, Kg. Melayu, 47000 Sungai Buloh, Selangor. Tel no.: 603 6126 1304 / 6012 201 6922. Fax no.: 603 6140 2249. E-mail : norizah_ismail@moh.gov.my

Madam Janagi Naidu, Science Officer, National Public Health Laboratory, Ministry of Health Malaysia, Lot 1853, Kg. Melayu, 47000 Sungai Buloh Selangor. Tel no.: 603 6126 1200 / 6016 701 7454. Fax no.: 6036140 2249. E-mail : janagi@moh.gov.my

Madam Rashidah binti Mohammad, Science Officer, National Public Health Laboratory, Kota Kinabalu, Ministry of Health Malaysia, Bukit Padang, Jalan Kolam, 88850 Kota Kinabalu, Sabah. Tel no.: 6088 250 710 / 6016 809 1076. Fax no.: 6088 243 210. E-mail : rashidah_mohd@sbh.moh.gov.my; rashidah1974@yahoo.com

MONGOLIA
Dr Rentsen Tuul, Head, National Measles Laboratory, National Center of Communicable Diseases, Baynzurkh District, Nam Yan Ju Street Ulaanbaatar. Tel no.: 976 1199093674. E-mail : r_tuul@yahoo.com

Dr Sambuu Anand, Surveillance Officer, EPI, Department of Immunization National Center of Communicable Diseases, Baynzurkh District, Nam Yan Ju Street, Ulaanbaatar. Tel no.: 976 9925 8021. E-mail : anand_sambuu@yahoo.com

NEW ZEALAND
Ms Sheryl Young, Section Head Virology/Serology, Canterbury Health Laboratories, corner Hagley Avenue and Tuam Street, P.O. Box 151 Christchurch. Tel no.: 64 03 3641 229. Fax no.: 64 03 03640 750 E-mail : sheryl.young@cdhb.health.nz

PAPUA NEW GUINEA
Dr Evelyn Lavu, Manager SSMO, Central Public Laboratories P.O. Box 1774, Boroko, National Capital District Tel no.: 675 72000247. E-mail : lavuek@gmail.com

Miss Janlyn Kumbu, National Surveillance Laboratory Scientific Officer-In-Charge, Central Public Health Laboratory Private Mail Bag 1, Boroko, National Capital District Tel no.: 675 3248199. Fax no.: 675 3256342 E-mail : kumbuj@gmail.com

Mr Edilson Yano, Paediatric Surveillance Officer, National Health Department P.O. Box 807, Waigani National Capital District, Tel no.: 675 3013730. Fax no.: 675 3256179. E-mail : eyano2012@gmail.com

PHILIPPINES
Dr Amado O. Tandoc III, Medical Specialist III, Research Institute for Tropical Medicine, 9002 Research Drive, FCC Compound, Alabang, Muntinlupa City 1781. Tel no.: 632 8098120. Fax no.: 632 8097120. E-mail : amado.tandocmd@gmail.com
Annex 2

PHILIPPINES
Mr Rex Centeno, Science Research Specialist II, Research Institute for Tropical Medicine, 9002 Research Drive, FCC Compound, Alabang, Muntinlupa City 1781. Tel no.: 632 8098120. Fax no.: 632 8097120. E-mail : rexcenteno@yahoo.com

Dr Maria Joyce Ducusin, Medical Specialist IV, National Center for Disease Prevention and Control, Building 14 San Lazaro Compound, Sta. Cruz, Manila. Fax no.: 632 7329956. Mobile no.: 63 9175725992. E-mail : juducusin@yahoo.com

REPUBLIC OF KOREA
Dr Kisoon Kim, Director Division of Respiratory Viruses, Center for Infectious Disease, NIH, Korea Centers for Disease Control and Prevention, OHTAC, 187 Osongsaemyeong2-ro, Osong-eup, Cheongwon-gun, Chungbuk 363-651. Tel no.: 82 43 719 8220. Fax no.: 82 43 719 8239 E-mail : tigerkis@nih.go.kr

SINGAPORE
Dr Lui Sook Yin, Scientific Officer, Singapore General Hospital Department of Pathology, #01-01B, Outram Road, Singapore 169608 Tel no.: 65 63265435. Fax no.: 65 63234972. Mobile no.: 65 91779985 E-mail : lusook.yin@sgh.com.sg

VIET NAM
Dr Nguyen Thi Hien Thanh, Vice Head, Virology Department Head, Enterovirus Laboratory, Manager of Measles and JE Laboratories National Institute of Hygiene and Epidemiology No. 1, Yersin Street, Hanoi 10000. Tel no.: 04 3 9726851 ext 218 Fax no.: 04 3 9726850. E-mail : thanhpoliolab@yahoo.com; thanh.enterovirus@nihe.org.vn

Dr Dinh Tuan Duc, Researcher, Respiratory Laboratory, Virology Department, National Institute of Hygiene and Epidemiology No. 1, Yersin Street, Hanoi 10000. Tel no.: 04 3 9726851 ext 107. Fax no.: 04 3 9726850. E-mail : ducvic2002@yahoo.com

Ms Pham Thi Nhung, Staff in Respiratory Laboratory, Pasteur Institute 167 Pasteur Street, District 03, Ho Chi Minh City. Tel no.: 2 43 719 8491. Fax no.: 82 43 719 8519. E-mail : ptnhung82@yahoo.com

Mrs Huynh Kim Mai, Researcher, Pasteur Institute in Nha Trang 8-10 Tran Phu Street, Nha Trang, Khanh Hoa. Tel no.: 84 98 847 9925. E-mail : mai064@yahoo.com

2. TEMPORARY ADVISERS

Dr Paul Rota, Chief, Measles Virus Section, Centers for Disease Control and Prevention, Mailstop C-22, 1600 Clifton Road, Atlanta, Georgia 30033, United States of America. Tel no.: 404 639 3512. Fax no.: 404 639 4178. E-mail : par1@cdc.gov
Annex 2

Dr Olen Martin Kew, Associate Director, Global Laboratory Science, National Center for Immunization and Respiratory Diseases, Division of Viral Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop C-22, Atlanta, Georgia 30333, United States of America. Tel no.: 404 639 3940. Fax no. 404 639 4011. E-mail : omk1@cdc.gov

Dr Michael Catton, Director, Victorian Infectious Diseases Reference Laboratory, Locked Bag 815, Carlton South, Victoria 3053, Australia. Tel no.: 61 3 9342 2636. Fax no.: 61 3 9342 2666. E-mail : mike.catton@mh.org.au

Dr Li Jin, Clinical Scientist, Microbiology Services, Centre for Infections, Health Protection Agency 61 Colindale Avenue, London NW9 5EQ, United Kingdom, Tel no.: 44 208 327 6045. E-mail : li.jin@hpa.org.uk

Dr Min-hsin Chen, Associate Service Fellow, Rubella Virus Section, Unites States Centers for Disease Control and Prevention, Mailstop C-22, 1600 Clifton Road, Atlanta, Georgia 30033, United States of America, Tel no.: 404 639 3508. E-mail : zvp8@cdc.gov

Dr Barbara Johnson, Diagnostic and Reference Laboratory, Arbovirus Diseases Branch Division of Vector-Borne Infectious Diseases (DVBID), Centers for Disease Control and Prevention (CDC), 3150 Rampart Road, Building 401, Room 3-322, Fort Collins, Colorado 80521, United States of America. Tel no.: 1970 266 3543. Fax no.: 1970 221 6441. E-mail : bfj9@cdc.gov

Dr Pei Yun Shu, Research and Diagnostic Center, Centers for Disease Control , 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2278 3992

Dr Ho-Sheng Wu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2783 7779

Mr Ricardo A. Oraya, Jr., Surveillance Officer for VPDs, National Epidemiology Center Department of Health, Building 19, 2nd Floor, San Lazaro Compound, Sta. Cruz, 1003, Manila Tel no.: 63 2 651 7800 local 2930. Mobile no.: 63 916 2643170. E-mail : ricardo.oraya@yahoo.com

Dr Dhanraj Samuel, Health Protection Agency, Microbiology Services Division-Colindale, Virus Reference Department, 61 Colindale Avenue, London NW9 5EQ, United Kingdom. Tel no.: 44 0 208 200 4400. E-mail : dhan.samuel@hpa.org.uk

Dr Jeong-Gu Nam, Deputy Scientific Director, Division of Respiratory Virus, Osong Health Technology Administration Complex, 187 Osongsaengmyeong2(i)-ro, Osong-eup Cheongwon-gun Chungbuk, 363-951, Republic of Korea. E-mail : jeonggu64@korea.kr

Dr Makoto Takeda, Department of Virology III, National Institute of Infectious Diseases, Toyama 1-23-, Shinjuku-ku, Tokyo 162-8640, Japan. E-mail : mtakeda@nih.go.jp

3. OBSERVERS

Dr Pei Yun Shu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Tel no.: 8862 2278 3992

Dr Ho-Sheng Wu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2783 7779

Mr Ricardo A. Oraya, Jr., Surveillance Officer for VPDs, National Epidemiology Center Department of Health, Building 19, 2nd Floor, San Lazaro Compound, Sta. Cruz, 1003, Manila Tel no.: 63 2 651 7800 local 2930. Mobile no.: 63 916 2643170. E-mail : ricardo.oraya@yahoo.com

Dr Dhanraj Samuel, Health Protection Agency, Microbiology Services Division-Colindale, Virus Reference Department, 61 Colindale Avenue, London NW9 5EQ, United Kingdom. Tel no.: 44 0 208 200 4400. E-mail : dhan.samuel@hpa.org.uk

Dr Jeong-Gu Nam, Deputy Scientific Director, Division of Respiratory Virus, Osong Health Technology Administration Complex, 187 Osongsaengmyeong2(i)-ro, Osong-eup Cheongwon-gun Chungbuk, 363-951, Republic of Korea. E-mail : jeonggu64@korea.kr

Dr Makoto Takeda, Department of Virology III, National Institute of Infectious Diseases, Toyama 1-23-, Shinjuku-ku, Tokyo 162-8640, Japan. E-mail : mtakeda@nih.go.jp
1. PARTICIPANTS

CAMBODIA

Mr Buth Sokhal, Deputy Director, National Institute of Public Health
Lot #2, Kim Yi Sung Boulevard, Sangkat Boeng Kok II, Khan Tuol Kork Phnom Penh, Tel no.: 855 16 888 889. Fax no.: 855 23 882 889.
E-mail: buthsokhal@yahoo.com

Mr Am Chanthan, Head of Immunology Unit (Laboratory)
National Institute of Public Health, Lot #2, Kim Yi Sung Boulevard
Sangkat Boeng Kok II, Khan Tuol Kork, Phnom Penh
Tel no.: 855 12 881 196. Fax no.: 855 23 882 889
E-mail: am.chanthan07@gmail.com

Mrs Ngeth Savry, Immunization Officer, National Immunization Programme, Ministry of Health, No. 6A Prek Leap Khan Russey Keo Phnom Penh. Tel no.: 855 12 679 896. Fax no.: 855 23 426 257.
E-mail: ngethsavry@yahoo.com.

CHINA

Dr Liang Guoqiong, Professor, Department of Viral Encephalitis Institute for Viral Disease Control and Prevention
155# Changbai Road, ChangPing District, Beijing 102206
Tel no.: 8610 5890 0838. Fax no.: 8610 5890 0839.
E-mail: gdliang@hotmail.com

Dr Wang Huanyu, Associate Researcher, Department of Viral Encephalitis Institute for Viral Disease Control and Prevention, 155# Changbai Road, ChangPing District, Beijing 102206. Tel no.: 8610 5890 0843 Fax no.: 8610 5890 0840. E-mail: rainoffall@yahoo.com
CHINA
Dr Yu Wenzhou, Professor, National Immunization Program
Chinese Center for Disease Control and Prevention, 27 Nanwei Road
Beijing. Tel no.: 8610 630279466. Fax no.: 8610 630279468.
E-mail : wenzhouyu69@hotmail.com.

Dr Tan Xiaojuan, Assistant Researcher, National Institute of Viral Disease
Control and Prevention, Chinese Center for Disease Control and Prevention
155# Changbai Road, Changping District, Beijing. Tel no.: 8610 58900185
E-mail : tan.xiao.juan@hotmail.com

JAPAN
Dr Tomohiko Takasaki, Chief, Laboratory of Vector-borne viruses
National Institute of Infectious Diseases, 1-23-1 Toyama Shinjuku-ku
Tokyo 162-8640. Tel no.: 81 35285 1111 ext. 2526.
Fax no.: 81 35285 1188. E-mail : takasaki@nih.go.jp

LAO PEOPLE'S
Dr Phengta Vongphrachanh Director National Center for Laboratory and
DEMOCRATIC
Epidemiology, Ministry of Health, Km3 Thadeua Road, Sisattanak District,
REPUBLIC
Vientiane Tel no.: 8 56 21 315858. Fax no.: 856 21 315858.
E-mail : v.phengta@gmail.com

Dr Darouny Phonekeo, Deputy Chief of Laboratory Department
National Center for Laboratory and Epidemiology, Ministry of Health
Km3 Thadeua Road, Sisattanak District, Vientiane.
Tel no.: 856 21 312351; 856 21 350209. Fax no.: 856 21 350209.
E-mail : darounyphonekeo@gmail.com

Mr Virasack Som Oulay, Staff of Sero-Virology Laboratory
National Center for Laboratory and Epidemiology, Ministry of Health
Km3 Thadeua Road, Sisattanak District, Vientiane. Tel no.: 856 21 312858.
Fax no.: 856 21 312351; 856 21 315347. E-mail : v.phengta@gmail.com

Dr Chansay Pathammavong, Deputy Manager of National Immunization
Program, Mother and Child Health Centre, Ministry of Health. Km3
Thadeua Road, Sisattanak District, Vientiane. Tel no.: 856 021 312352.
Fax no.: 856 021 312120. E-mail : chansay_epi@yahoo.com

MALAYSIA
Madam Norizah binti Ismail, Science Officer, National Public Health
Laboratory, Ministry of Health Malaysia, Lot 1853, Kg. Melayu,
47000 Sungai Buloh, Selangor. Tel no.: 603 6126 1304 / 6012 201 6922.
Fax no.: 603 6140 2249. E-mail : norizah_ismail@moh.gov.my

PAPUA NEW
Dr Evelyn Lavu, Manager, SSMO Central Public Laboratories,
GUINEA
P.O. Box 1774, Boroko, National Capital District, Tel no.: 675 72000247.
E-mail : lavuek@gmail.com
Annex 2

**PAPUA NEW GUINEA**
Miss Janlyn Kumbu, National Surveillance Laboratory, Scientific Officer-In-Charge, Central Public Health Laboratory, Private Mail Bag 1, Boroko National Capital District. Tel no.: 675 3248199. Fax no.: 675 3256342. E-mail: kumbuj@gmail.com
Mr Edilson Yano, Paediatric Surveillance Officer, National Health Department, P.O. Box 807, Waigani, National Capital District, Tel no.: 675 3013730. Fax no.: 675 3256179. E-mail: cyano2012@gmail.com

**PHILIPPINES**
Dr Amado O. Tandoc III, Medical Specialist III, Head, Virology Department, Research Institute for Tropical Medicine, 9002 Research Drive, FCC Compound, Alabang, Muntinlupa City 1781. Tel no.: 632 8098120. Fax no.: 632 8097120. E-mail: amado.tandocmd@gmail.com

Dr Maria Joyce Ducusin, Medical Specialist IV, National Center for Disease Prevention and Control, Building 14 San Lazaro Compound, Sta. Cruz, Manila. Fax no.: 632 7329956. Mobile no.: 63 9175725992. E-mail: juducusin@yahoo.com

**REPUBLIC OF KOREA**
Dr Myung Guk Han, Deputy Scientific Director, Division of Arboviruses, National Institute of Health, Korea Centers for Disease Control and Prevention, 187 Osongsaemyeong2-ro, Osong-eup, Cheongwon-gun, Chungbuk 363-651. Tel no.: 82 43 719 8491. Fax no.: 82 43 719 8519. E-mail: mghan@korea.kr

**VIET NAM**
Dr Nguyen Thi Hien Thanh, Vice Head, Virology Department Head, Enterovirus Laboratory, Manager of Measles and JE Laboratories National Institute of Hygiene and Epidemiology, No. 1, Yersin Street, Hanoi 10000. Tel no.: 04 3 9726851 ext 218. Fax no.: 04 3 9726850 E-mail: thanhpoliolab@yahoo.com; thanh.enterovirus@nihe.org.vn

Dr Nguyen Thi Thu Thuy, Head, Arbovirus Laboratory, National Institute of Hygiene and Epidemiology, No. 1 Yersin Street, Hanoi. Tel no.: 04 3 9726851 ext. 211. Fax no.: 04 3 9726850. E-mail: ticun_2002@yahoo.com

Dr Huynh Phuong Thao, Researcher, Pasteur Institute of Ho Chi Minh City 167 Pasteur Street, District 3, Ho Chi Minh City. Tel no.: 84 8 38 296351. Fax no.: 84 8 38 231519. E-mail: huynhthao196@yahoo.com
2. TEMPORARY ADVISERS

Dr Barbara Johnson, Diagnostic and Reference Laboratory, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases (DVBID), Centers for Disease Control and Prevention (CDC), 3150 Rampart Road, Building 401, Room 3-322, Fort Collins, Colorado 80521, United States of America. Tel no.: 1970 266 3543. Fax no.: 1970 221 6441. E-mail : bfj9@cdc.gov

Dr Ichiro Kurane, Deputy Director-General, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Tel no.: 81 3 5285 1111 ext. 2003. Fax no.: 81 3 5285 1356. E-mail : kurane@niid.go.jp; kurane@nih.go.jp

Mrs Anisone Chanthongthip, Laboratory Manager, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People's Democratic Republic. Tel no.: 856 21285119. E-mail : anisone@tropmedres.ac

Dr Olen Martin Kew, Associate Director, Global Laboratory Science, National Center for Immunization and Respiratory Diseases, Division of Viral Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop C-22, Atlanta, Georgia 30333, United States of America. Tel no.: 404 639 3940. Fax no. 404 639 4011. E-mail : omk1@cdc.gov

Dr Min-hsin Chen, Associate Service Fellow, Rubella Virus Section, Unites States Centers for Disease Control and Prevention, Mailstop C-22, 1600 Clifton Road, Atlanta, Georgia 30033, United States of America, Tel no.: 404 639 3508. E-mail : zvp8@cdc.gov

Dr Li Jin, Clinical Scientist, Microbiology Services, Centre for Infections, Health Protection Agency 61 Colindale Avenue, London NW9 5EQ, United Kingdom, Tel no.: 44 208 327 6045. E-mail : li.jin@hpa.org.uk

3. OBSERVERS

Dr Pei Yun Shu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2278 3992

Dr Ho-Sheng Wu, Research and Diagnostic Center, Centers for Disease Control, 161 Kun-Yang Street, Nan-King, Taipei 11561. Fax no.: 8862 2783 7779

Mr Ricardo A. Oraya, Jr., Surveillance Officer for VPDs, National Epidemiology Center Department of Health, Building 19, 2nd Floor, San Lazaro Compound, Sta. Cruz, 1003, Manila Tel no.: 63 2 651 7800 local 2930. Mobile no.: 63 916 2643170. E-mail : ricardo.oraya@yahoo.com

Dr Raj Shankar Ghosh, Technical Director, India Country Program, Program for Appropriate Technology in Health, A-9, Qutab Institutional Area, U.S.O. Road, New Delhi 110067, India. Tel no.: 91 11 2653 0080/88. Fax no.: 91 11 2653 0089. E-mail : rghosh@Path.org
Annex 2

Dr Lea Necitas Apostol, National Polio Laboratory Technical Supervisor, Research Institute for Tropical Medicine, Filinvest Corporation City Compound, Alabang, Muntinlupa City 1781 Philippines. Tel no.: 632 8072628. E-mail : leinecitas9780@yahoo.com

Ms Ava Kristy D. Sy, National JE Laboratory Technical Supervisor, Research Institute for Tropical Medicine, Filinvest Corporation City Compound, Alabang, Muntinlupa City 1781, Philippines. Tel no.: 632 8072628. E-mail : avakristysy@gmail.com

3. SECRETARIAT

Dr John Patrick Ehrenberg, Director, Combating Communicable Diseases, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 8001. Fax no.: 63 2 521 1036. E-mail : ehrenberg@wpro.who.int

Dr Sergey Diorditsa, Team Leader, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue, 1000 Manila. Tel no.: 63 2 528 9045. Fax no.: 63 2 521 1036. E-mail : diorditsas@wpro.who.int

Dr Youngmee Jee, Scientist, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9744. Fax no.: 63 2 521 1036. E-mail : jeey@wpro.who.int

Ms Liliane Boualam, Technical Officer, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9741. Fax no.: 63 2 521 1036. E-mail : boualam1@wpro.who.int

Dr Wang Xiaojun, Medical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9747. Fax no.: 63 2 521 1036. E-mail : wangx@wpro.who.int

Dr William Schluter, Medical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9748. Fax no.: 63 2 521 1036. E-mail : schluterw@wpro.who.int

Dr Kimberley Fox, Technical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9033. Fax no.: 63 2 521 1036. E-mail : foxk@wpro.who.int

Dr Fem Julia Paladin, Technical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9737. Fax no.: 63 2 521 1036. E-mail : paladinf@wpro.who.int

Dr Karen Hennessey, Technical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9034. Fax no.: 63 2 521 1036. E-mail : hennesseyk@wpro.who.int
Mr Gabriel Anaya, Programme Management Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9740. Fax no.: 63 2 521 1036. E-mail: anayag@wpro.who.int

Dr Jorge Mendoza Aldana, Technical Officer, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9751. Fax no.: 63 2 521 1036. E-mail: mendozaaldanaj@wpro.who.int

Mr Benjamin Bayutas, Informatics Assistant (Data Management), Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9739. Fax no.: 63 2 521 1036. E-mail: bayutasb@wpro.who.int

Ms Kayla Mariano, Informatics Assistant (Data Management), Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, United Nations Avenue 1000, Manila. Tel no.: 63 2 528 9738. Fax no.: 63 2 521 1036. E-mail: marianok@wpro.who.int

Mr John Hustedt, STOP Team Member, World Health Organization, No. 177-179 corner Streets Pasteur(51) and 254, Sankat Chak Tomouk, Khan Daun Penh, Phnom Penh, Cambodia. Tel no.: 855 23 216610. Fax no.: 855 23 216211. E-mail: steepi@cam.wpro.who.int

Mr Alejandro Ramirez-Gonzalez, Technical Officer (VPD Surveillance), Expanded Programme on Immunization, WHO Representative Office in Laos, Ban Phoxay, That Luang Road, Vientiane, Lao People's Democratic Republic. Fax no.: 856 21 353-905. E-mail: gonzaleza@wpro.who.int

Dr Siddhartha Datta, Technical Officer, Expanded Programme on Immunization, WHO Representative Office in Papua New Guinea, 4th Floor, AOPI Centre, Waigani Drive, Port Moresby, Papua New Guinea. Tel no.: 67 5 325 7827. Fax no.: 67 5 325 0568. E-mail: dattas@wpro.who.int

Dr Sigrun Roesel, World Health Organization in the Philippines, National Tuberculosis Centre Building, Second Floor, Bldg. 9, Department of Health, San Lazaro Hospital Compound Sta. Cruz, Manila. Tel no.: 63 2 528 9761. Fax no.: 63 2 731 3914. E-mail: roesels@wpro.who.int

Dr Kohei Toda, Medical Officer, Expanded Programme on Immunization, WHO Representative Office in Viet Nam, 63 Tran Hung Dao Street, Hoan Kiem District, Hanoi. Tel no.: 844 3 943 3734. Fax no.: 844 3 943 3740. E-mail: todak@wpro.who.int

Dr Ousmane Diop, Global Polio Laboratory Coordinator, Surveillance, Data and Certification World Health Organization, Avenue Appia 20, CH 1211, Geneva 27, Switzerland. Tel no.: 41 22 79 12503. Fax no.: 41 22 79 13111. E-mail: diopo@who.int

Dr Miguel Norman Mulders, Scientist, Expanded Programme on Immunization, World Health Organization, Avenue Appia 20, CH 1211, Geneva 27, Switzerland. Tel no.: 41 22 79 14405. Fax no.: 41 22 79 13111. E-mail: muldersm@who.int