Training Workshop Report

The Fifth Regional Hands-on Training Workshop on the Laboratory Diagnosis of Measles and Rubella

Hong Kong (China)
29 October - 3 November 2012

World Health Organization
Western Pacific Region
REPORT

THE FIFTH REGIONAL HANDS-ON TRAINING WORKSHOP
ON THE LABORATORY DIAGNOSIS OF MEASLES AND RUBELLA

Convened by:

WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

Hong Kong (China)
29 October to 3 November 2012

Not for sale

Printed and distributed by:

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

April 2013
NOTE

The views expressed in this report are those of the participants of the Fifth Hands-on Training on the Laboratory Diagnosis of Measles and Rubella and do not necessarily reflect the policies of the World Health Organization.

This report has been prepared by the World Health Organization Regional Office for the Western Pacific for the participants of the 5th Hands-on Training on the Laboratory Diagnosis of Measles and Rubella, which was held in Hong Kong (China) from 29 October to 3 November 2012.

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

The Fifth Hands-on Training on the Laboratory Diagnosis of Measles and Rubella was held at the Public Health Laboratory Centre (PHLC) in Hong Kong (China) from 29 October to 3 November 2012.

The training was organized by the Expanded Programme on Immunization (EPI) of the WHO Regional Office for the Western Pacific, and was hosted by the Virology Division, Centre for Health Protection, Hong Kong (China).

The training was attended by 13 participants from WHO-designated national or regional reference measles / rubella laboratories from Australia (one participant), China (three participants), Malaysia (one participant), Mongolia (one participant), New Zealand (one participant), the Philippines (two participants), Singapore (one participant), the Republic of Korea (one participant) and Viet Nam (two participants). In addition to WHO Secretariat, the training was facilitated by temporary advisers from the United States Centers for Disease Control and Prevention (US CDC), National Institute of Infectious Diseases (NIID), Japan, and Public Health Laboratory Centre (PHLC), Hong Kong (China).

The objectives of the workshop were:

1. To enhance the knowledge and skills of staff from WHO regional reference laboratories and selected national measles and rubella laboratories in:
   
   a. molecular detection of measles and rubella viruses using the new real-time reverse transcriptase polymerase chain reaction (rRT-PCR) and sequencing;

   b. laboratory quality assurance of molecular detection of measles and rubella virus; and

   c. sequence data analysis of measles and rubella data analysis.

2. To discuss and practice the procedures of depositing the measles and rubella genotype and sequence data to measles nucleotide surveillance (MeaNS) and the WHO genotype database.

The training, which consisted of lectures, country reports and practical sessions, focused on understanding the needs and role of the measles and rubella laboratory networks and learning about the use of molecular assays. During the practical session, real-time RT-PCR, conventional PCR and sequencing for virus genotyping for measles and rubella were introduced to the participants.

Participants received practice molecular proficiency test (PT) samples at the end of the training and were requested to complete PT and report results to US CDC and WHO regional laboratory coordinator within six weeks using standardized reporting format. Most of the participants provided the PT results within the agreed time. US CDC provided comprehensive feedback to the countries on their PT results.
Overall, the participants provided positive feedback on the workshop, which they considered met its objectives. Participants also expressed that the schedule and administrative arrangements were well organized.
1. INTRODUCTION

In 2003, the WHO Regional Committee for the Western Pacific declared a measles elimination goal and in 2005, established a target date of 2012 for regional elimination. As a result, the Measles and Rubella Laboratory Network (LabNet) for the Western Pacific Region, consisting of one global specialized laboratory (GSL) and three regional reference laboratories (RRLs), was established, with 16 national and 362 subnational laboratories in China. The WHO Measles and Rubella Laboratory Network play a critical role in monitoring the progress of measles elimination and rubella control. The roles of laboratories in the network include confirming measles and rubella cases, and providing genotyping or molecular data to better understand the epidemiology of measles. Timeliness in providing reliable laboratory data is critical to identify and respond to imported or endemic measles transmission, especially since the Region is approaching measles elimination.

Following the Global Poliomyelitis Laboratory Network model, a WHO accreditation system for measles and rubella laboratories was developed at the global level to ensure the performance quality of network laboratories. To ensure quality assurance, WHO proficiency testing and confirmatory testing programmes have been established.

Detection of measles or rubella immunoglobulin (IgM) in serum is the standard test for the laboratory diagnosis of measles and rubella. However, the role of the measles and rubella laboratory network also extends to molecular surveillance, and laboratories with the capacity for virus isolation, molecular diagnosis and sequencing are encouraged to conduct this type of surveillance. Establishing baseline genetic data on measles viruses that are currently circulating in the Region will allow differentiation between importation and indigenous circulation of measles virus strains. Genotype and sequence information are submitted to the WHO genotype and MeaNS database by national or regional laboratories. Genotype data on recent measles virus strains are available from most countries, except from Pacific island countries and areas (PICs).

In 2010, the Fourth Regional Hands-on Training Workshop on the Laboratory Diagnosis of Measles and Rubella focusing on Molecular Diagnosis was held at the regional reference laboratory (RRL) in Hong Kong (China). This workshop aimed to enhance the knowledge and skills of laboratory staff in molecular detection of measles and rubella viruses by reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing and to enhance laboratory quality assurance of measles and rubella diagnosis. Participants from China, Japan, Malaysia, Mongolia, New Zealand, the Philippines, the Republic of Korea, Singapore, and Viet Nam attended this workshop.

The Fifth Regional Hands-on Training Workshop on the Laboratory Diagnosis of Measles and Rubella took place over five and a half days. It aimed to further strengthen the capacity for the molecular detection of measles and rubella viruses using the new real-time RT-PCR (rRT-PCR) as well as conventional RT-PCR and sequencing capacity as a follow-up to the previous hands-on training. The WHO network laboratories that would benefit from training on molecular detection and sequencing were invited. At the end of the workshop, participants were requested to carry practice molecular proficiency test (PT) samples for measles and rubella. All laboratories were requested to test these samples and to report results to the WHO Regional Office for the Western Pacific within six weeks after the samples were received in the laboratory.
1.1 Objectives

(1) To enhance the knowledge and skills of staff from WHO regional reference laboratories and selected national measles and rubella laboratories in:

(a) molecular detection of measles and rubella viruses using the new real-time reverse transcriptase polymerase chain reaction (rRT-PCR) and sequencing;

(b) laboratory quality assurance of molecular detection of measles and rubella virus; and

(c) sequence data analysis of measles and rubella data analysis.

(2) To discuss and practice the procedures of depositing the measles and rubella genotype and sequence data to measles nucleotide surveillance (MeaNS) and the WHO genotype database.

1.2 Participants

The training was attended by 13 participants from WHO-designated national or regional reference measles or rubella laboratories from Australia (one participant), China (three participants), Malaysia (one participant), Mongolia (one participant), New Zealand (one participant), the Philippines (two participants), the Republic of Korea (one participant), Singapore (one participant) and Viet Nam (two participants). In addition to WHO Secretariat, the training was facilitated by temporary advisers from the United States Centers for Disease Control and Prevention (US CDC), National Institute of Infectious Diseases (NIID), Japan, and Public Health Laboratory Centre (PHLC), Hong Kong (China). A list of participants and facilitators is included in Annex 1.

1.3 Opening Remarks

Dr Thomas Tsang from the PHLC in Hong Kong (China) welcomed the participants and gave the introductory speech to open the hands-on training workshop.

2. PROCEEDINGS

2.1 Lecture sessions

2.1.1 Updates from the WHO Regional Office for the Western Pacific measles and rubella laboratory network and objectives of the training

Dr Youngmee Jee, Scientist, EPI, WHO Western Pacific Regional Office provided updates on the Measles and Rubella Laboratory Network in the Western Pacific Region and discussed the objectives of the training. She mentioned that during the Regional Committee meeting in 2010, some WHO regional offices adopted the goal to eliminate measles by 2015. In every WHO region beginning in 2008, monthly measles incidence experienced a decreasing trend, with the lowest incidences in 2012. The targeted disease control initiatives of the Expanded Programme on Immunization of the WHO Regional Office for the Western Pacific (which are measles elimination, Hepatitis B control, maintenance of polio-free status and maternal/neonatal tetanus elimination in five remaining countries, were reviewed. Several key indicators of progress
towards measles elimination were discussed, such as the overall vaccination coverage in 2011 for measles containing virus 1 (MCV1) (96% coverage) and measles containing virus 2 (MCV2) (91% coverage). It was reported that in 2010–2011, 134 million people were immunized through measles supplementary immunization activities in eight countries, namely: Cambodia, China, Federated States of Micronesia, Lao People's Democratic Republic, Philippines, Papua New Guinea, and North and South Viet Nam, with either measles monovalent or measles rubella (MR) vaccine. In 2012, Papua New Guinea and Solomon Islands completed its supplementary immunization activities (SIAs) and Mongolia is planning in October targeting children 2-14 years. Despite the recent outbreaks in Malaysia and the Philippines, there was a remarkable progress made in measles elimination in the Western Pacific region that demonstrates measles elimination is achievable in the Region.

The performance of the Measles and Rubella Laboratory Network was also reviewed. It was reported that 96% (48 out of 50) of laboratories in the network were accredited as of October 2013. The two laboratories that were not accredited were one national laboratory in the Lao People's Democratic Republic and one subnational laboratory in Xinjiang, China. All but four of the measles and rubella laboratories are able to perform virus isolation, PCR, sequencing and genotyping. The four laboratories are those in Cambodia, Fiji, the Lao People's Democratic Republic, and Papua New Guinea. A WHO Microsoft SharePoint database currently serves as a repository for measles and rubella genotypes until all countries are submitting the genotype information to the database (measles nucleotide surveillance (MeaNS) and rubella nucleotide surveillance (RubeNS). As of June 2012, 74 countries reported eight measles genotypes: B2, B3 D4, D8, D9, D11, G3 and H1. In 2011, 13 countries reported five rubella genotypes: 1E, 1G, 1j, 1h and 2B. Confirmatory testing and concordance rates of most laboratories were higher than 90%. Dr Jee also reviewed the recommendations from the third laboratory network meeting held in September 2011.

2.1.2 Importance of molecular epidemiology and genotype information of measles virus

Dr Paul Rota, Chief, Measles Virus Section, Centers for Disease Control and Prevention USA, presented on the use of real-time and conventional RT-PCR for case classification and molecular surveillance of measles virus. Molecular epidemiologic studies are a key component of verification of measles elimination that target to obtain genotype information from at least 80% of chains of transmission. The criterion for elimination of measles is absence of an endemic genotype for one year. Genetic data in conjunction with standard epidemiologic information can be used to track transmission patterns and identify sources of infection. Only sequence analysis can distinguish vaccine reactions from infection with wild type virus. Dr Rota discussed the status of measles in the United States of America and the two imported cases reported from the Dominican Republic and Canada. D4 measles genotype was detected from one of those cases.

Dr Rota emphasized the advantages of using real-time RT-PCR (rRT-PCR). It can detect 10-100 copies of RNA/sample in a high throughput format and produce results within two hours. It also can help to confirm a case when serologic results are inconclusive but negative results do not rule out a case. It is more sensitive than conventional (endpoint) RT-PCR. Sequence information from the conventional PCR is required for genotype assignment and confirmation of vaccine reactions. The real-time PCR product is not suitable for sequence analysis. The measles rRT-PCR kits supplied by US CDC contain primers and probes only, other reagents and materials need to be supplied by the user. Measles genotyping (conventional) RT-PCR improved primers MeV214 and MeV216 are designed to amplify a 634 nucleotide region coding for the 3’ terminus of the nucleoprotein (N) gene in a conventional RT-PCR reaction. They were tested to detect 11 different genotypes. Version 2.0 of the measles genotyping kit contains stocks of the improved primers MeV214/MeV216 and the new synthetic control RNA (MeV-N3in). The primers are also used in the sequencing reactions.
2.1.3 Introduction of molecular technique for rubella virus detection and genotyping by RT-PCR and real-time RT-PCR

Dr Paul Rota discussed the molecular approaches for rubella virus using real-time and genotyping RT-PCR. Molecular testing is used in improving case classification in the first three days after rash onset, monitoring infectivity of congenital rubella syndrome in infants, distinguishing between vaccine and wild-type viruses, establishing endemic genotype baselines and tracking transmission patterns and identifying sources of infection using genetic data. In pre- or post-elimination settings, the goal is to obtain genetic information from every chain of transmission, and specimens must be taken at first contact with suspected case. Dr Rota also presented the use of US CDC molecular assays for rubella diagnostic RT-PCR kit, rubella diagnostic real-time RT-PCR kit, and rubella virus genotyping kit. Processing of the sample for ribonucleic acid (RNA) extraction and virus isolation, and testing scheme for detection of rubella “direct from sample” molecular tests were discussed. The US CDC real-time RT-PCR kit for rubella virus RNA detection can detect as low as 10–30 copies of viral RNA with results in about four hours. The kit contains reagents for performing a qualitative assay using TaqMan chemistry. Rubella primers contain a mixture of two versions of the reverse primer needed to detect both clade 1 and clade 2 viruses. When performing rRT-PCR to detect rubella virus RNA, the test must be done properly with Ribonuclease P (RNase P), negative controls and positive controls. Negative results cannot be used to rule out a case because the negative result may be due to an inadequate specimen. US CDC also supplies conventional diagnostic RT-PCR to detect rubella virus RNA which is not as sensitive as the real-time assay, but serves as an acceptable alternative if real-time technology is not available. There are also challenges for sequencing/genotyping rubella viruses directly from samples because of very few conserved nucleotide sequence in envelope protein (E1), high guanine- cytosine (GC) content, low copy number in clinical samples, and large amplicon/sequence window size. Dr Rota also presented the rubella practice panel on fast technology for analysis of nucleic acids (FTA) elute cards that will serve as a practice panel for RNA extraction, rRT-PCR and genotyping. Global distribution of rubella genotypes from January 2007 to March 2012 showed that genotypes 1E, 1G and 2B were mostly found in all countries with few 1a, 1b, 1h and 1j, while, 1C, 1D, 1F, 1i, 2A, and 2C were inactive during this period.

2.1.4 Quality control for molecular tests

Dr Paul Rota presented the importance of quality control for molecular tests. Molecular characterization of measles and rubella viruses plays an increasingly important role in laboratory surveillance. It is therefore necessary to develop a quality control programme for molecular techniques. US CDC offers diagnostic RT-PCR and genotyping kits with positive controls and FTA practice panels to support quality control in the laboratory network. The protocols and kits for RT-PCR were validated with defined lower limit of detection, demonstrated specificity, and minimal background banding. The kits include control RNA of known sequence preferably with genetic markers to clearly identify control reactions, defined/optimized reaction conditions, demonstrated ability to detect all circulating genotypes and flexible platform/chemistry. He discussed strategies for working with RNA and how to avoid contamination. There should be dedicated equipment, rooms and hoods for all pre-amplification procedures and post-amplification analysis, use of filter tips for all pre-PCR procedures and for setting-up RT-PCR reactions, storage of eluted RNA at -20ºC or -70ºC, ice for working with RNA, frequent change gloves and avoidance of repeated freeze-thawing of RNA to prevent RNA degradation. The agarose gel electrophoreses results should clearly show molecular weight markers and document all agarose gels using photographs. PCR products for sequencing can be shipped at 4ºC or at room temperature. PCR products are stable at 4ºC, room temperature or 37ºC for one month. Considerations for a successful sequencing are as follows: template quality and quantity (check purified PCR product on a gel before sequencing), primer quality (repeated
freeze-thaws may affect primer quality) and chromatogram (should have evenly spaced, sharp and well defined peaks). Practice panel (measles or rubella infected cells) with Whatman fast technology for analysis of nucleic acids (FTA) cards was presented. FTA cards can be used for shipping virus isolates (but not serum samples) and can be stored and shipped at room temperature, reducing shipping costs. The results of FTA practice panels for measles and rubella were reported in a timely manner. The genotyping RT-PCR assays were performed well and almost all laboratories reported the correct results with minimal cross-contamination in standard, endpoint RT-PCR assays. All laboratories correctly identified the genotypes. There were no problems with stability during shipment.

2.1.5 Data Reporting, MeaNS/RubeNS database and GenBank

Dr Paul Rota’s presentation was based on a presentation by Mr Kevin E Brown from the Health Protection Agency (HPA), London, the United Kingdom of Great Britain and Northern Ireland. Dr Rota introduced the use and functionality of the measles nucleotide surveillance (MeaNS) database, as well as its content and the steps to use it online. The MeaNS database stores measles sequences and genotypes, is used to compare/phylogeny with viruses in other countries, and has the ability to upload to gene bank (GenBank) and report to WHO. The MeaNS database eliminates the need for multiple reporting. Only members of the WHO measles and rubella global laboratory network (including the Pan American Health Organization) can submit and view sequence data, according to the terms and conditions. Some information from the database may be downloaded without registration. Dr Rota also discussed registration to the database and submission of sequence files. Sequences can be stored according to WHO name but there cannot be two different sequences with same WHO name. The WHO name cannot be edited once submitted, however, if needed, an e-mail can be sent to MeaNS to modify the name. MeaNS will create WHO name when new sequences are submitted. GenBank is a genetic sequence data base hosted by National Institute of Health (NIH) USA. It is an annotated collection of all publicly available DNA sequences. A similar database on rubella nucleotide surveillance (RubeNS) is being developed. Examples of measles sequences from PAHO were presented. The MeaNS database is hosted by HPA and can be accessed on www.who-measles.org.

2.1.6 Instructions for FTA panels

Dr Paul Rota discussed the process and reporting of practice panels. The panel contains FTA discs loaded with lysates of measles or rubella infected cells. Filters are non-infectious but RNA remains intact and can be extracted by standard methods and can be used for RT-PCR, sequencing and sequence analysis. PCR products should be sequenced by the laboratories that are capable of sequencing or shipped to the appropriate RRL for sequencing analysis. The FTA cards/panel can be shipped at 4°C or at room temperature. RNA extraction procedure was presented using the QiaAmp Viral RNA Mini Kit. It was emphasized that measles RNA is stable for up to six months at ambient temperature, whereas, rubella control E1eMyc RNAs from cells are stable on FTA cards for one month at various temperatures. The participants were informed that the WHO practice panels will be distributed on the last day of the workshop. Mechanics on the processing, testing and reporting of practice panel results after the workshop were elaborated. Laboratories should follow correct reporting by using standard report forms, including all requested files, indicating "PAHO Practice Panel" as the subject of the email, and using appropriate e-mail addresses.

2.2 Country reports

2.2.1 Australia
Mr Thomas Tran from the Victorian Infectious Diseases Reference Laboratory (VIDRL) reported on importation of measles viruses (MeV) into Australia during January to September 2012 and the role of VIDRL in measles surveillance. The laboratory is using the measles virus (MeV) real-time PCR modified protocol of Hummel KB, Lowe L, Bellini WJ, Rota PA. J Virol Methods, 2006. RT-PCR testing strategy for measles virus genotyping was discussed. From January to September 2012, there were 285 samples tested using real-time PCR. Of the samples tested, 95 samples from 72 cases were positive and 190 were negative. Among the 72 positive cases, 45 were genotyped. There were six genotypes identified during the period namely: A (one), D8 (38), G3 (one), D9 (four), D4 (one), and B3 (three). The dominant circulating genotype was D8 which was widely spread in all areas except in South Australia. The highest number of varying measles virus genotypes was reported in New South Wales. The measles outbreak in New South Wales was the largest in 14 years (163 notifications) and began following importation from Thailand. Children up to four years old were those most affected (n=18), with 20%–30% of these children were below one year of age. In addition, high notification rates were observed among the 10–14 and 15–19 age groups and most of the cases were Australian born and under-immunized. Other importations of measles were from Indonesia, Myanmar, New Zealand and Uganda.

The measles vaccine was widely available in Australia in the early 1970s. People who missed vaccination during that time are now older than 40 years of age; however, the PCR results on measles cases showed that the clustering of cases is among people younger than 44 years of age. This suggests that some people younger than 44 years old still missed the measles vaccination. It may be that these people are migrants and denied receiving the vaccine. As a result, they may be under or unvaccinated and potentially fuelling transmissions and impacting herd immunity.

VIDRL was able to test 132 samples using the rubella virus real-time PCR (in-house design using E1 gene). Results showed that 127 samples were negative and five were positive which came from three individuals. One of the three individuals was exposed to a measles importation from Indonesia or Singapore, and was unvaccinated. Another individual was exposed to infection in India, and vaccination status was unknown. The third individual was a congenital rubella syndrome (CRS) case which resulted in medical termination of pregnancy. It was concluded that there is a diverse range of importations into a country that has a history of interrupted transmission, and under or unvaccinated people contribute to the transmissions and influencing the herd immunity.

2.2.2a China

Dr Yan Zhang from the Chinese Centers for Disease Control and Prevention presented the progress of measles elimination in China, the measles and rubella laboratory network and serologic testing, quality control, and measles and rubella viruses surveillance. China has made a lot of progress towards the elimination goal since 2009. To reach the elimination goal, the province-specific supplementary immunization activities (SIAs) were conducted during 2003–2009, and the synchronized nationwide SIAs were conducted in 2010. Based on the results of SIAs conducted, the last three consecutive from 2009 to 2011 had historically low incidence rates of measles. In terms of measles vaccination coverage, both the one-dose (MCV1) and two-dose (MCV2) vaccines have been achieving more than 95% coverage since 2001.
There were SIAs conducted in 27 provinces between 2003 to 2009, which resulted in a 60% decrease in cases in 2009 compared to 2008. After the nationwide SIAs in 2010, the number of cases in 2011 decreased by 74% with SIAs conducted in more than 400 counties with relatively high epidemic intensity. In 2012, during the “4.25 vaccination information campaign week”, selective SIAs were conducted across the country, resulted in a 72% decrease in the number of cases relative to the 2011 figure. There was a decreasing trend of confirmed measles cases by month of onset observed during 2007–2012. The numbers of outbreaks, measles cases and even the duration of outbreaks have been decreasing. The latest figures show that from January to June 2012, there were only seven measles outbreaks and a total of 15 cases, and ranges from zero to nine days of occurrences.

The China measles laboratory network is composed of three different levels of laboratories. Most of the serologic diagnosis for suspected measles cases is performed by prefecture laboratories. These laboratories are also responsible for the specimen collection, while the provincial laboratories perform virus isolation and identification and the national laboratories perform genotyping. Both the national and provincial laboratories are responsible to provide technical support and ensure and monitor the quality control implemented in the lower-level laboratories.

For serological tests, both imported and local kits are being used in Mainland China. Imported kits include Virion Serion for both measles and rubella while local kits include Haitai for measles and Kerunda for rubella. The proportion of the positive measles has decreased yearly and was about 15% measles cases and 21% rubella cases in 2012. With 40% of positive cases, the problem was determining the cause of the remaining negative cases with fever and rash. Establishing a fever and rash syndrome pathogenic surveillance is being considered to identify other etiologic agents. For sporadic cases in 2011, more than 32 000 serum samples were tested for measles IgM and almost 28 000 were tested for rubella IgM. Of these samples, 27% were measles positive and 18% were rubella positive. In 2012, more than 24 000 serum samples were tested for measles and almost 24 000 for rubella. Out of these samples tested, 13% were measles positive and 21% were rubella positive. Generally, majority of the samples tested has been classified as fever and rash cases with unknown pathogen. Cases from outbreaks comprise a very small portion of the total measles and rubella cases both in 2011 and 2012.

China has been receiving the PT panel from WHO Regional Office for the Western Pacific (WPRO) since 2009. The national and 31 provincial laboratories participate in annual proficiency tests with 100% score. For confirmatory testing, 31 provinces send serum panels to the national laboratory, which is responsible for reporting the results back to the provincial laboratories within 14 days upon receipt. The in-house control (IHC) for enzyme-linked immunosorbent assay (ELISA) was introduced into the China measles/rubella laboratory network to monitor variation between runs since 2006. Recent results showed a mean of 1.26 with +two standard deviation (2SD). WHO on-site accreditation review and hands-on training courses and workshops were conducted in 2011 and 2012.

From 1993 to 2012, 2452 isolates were obtained and six genotypes were detected such as: H1, H2, A, D4, D9, and D11. Of these genotypes, H1 is the predominant strain circulating for at least 20 years. There were also imported genotypes found since 2009. Except for Tibet, all other 30 provinces have obtained measles viruses since 1993 and all of the laboratories have detected H1 genotype. The imported genotypes H2, D4, D9 and D11 have been found in five provinces. The genotyping results of MeV in 2011 showed 294 isolates from 25 provinces with 288 H1a, three vaccine, and three D11. Genotyping results of MeV in 2012 showed 179 isolates from 16 provinces with 172 H1a, one vaccine, and six D9.
The incidence for rubella has been higher compared to that for measles since 2009. From January to June 2012, 32,785 cases have been reported and the rubella incidence rate is 2.45 per 100,000 population. This rate is 38.65% lower than the rubella incidence rate during the same period in 2011. Most of the cases reported were among those younger than 15 years old. The proportion of cases among those 15–39 years old has increased since 2004, and maintained the same level (about 40%) from 2007–2010. From 1999 to 2012, 843 rubella genotypes were obtained and 1E was the predominant genotype since 2001. The 2B genotype tends to be widespread in China. The 1E genotype was found in 27 provinces while 2B genotype was isolated in 13 provinces. Notably, 2B virus in 2011 was different from that in 2008 but with similarity of 95.9%, indicating a different transmission link. In 2012, both 1E and 2B genotypes were detected and two lineages were clustered for 2B virus. One of lineages came from the 2011 2B virus, which continues to circulate in different provinces and interprovince. The other lineage is new, with proportion (p)-distance of 0.033.

There has been substantial and rapid progress toward the elimination of measles in China. The China measles and rubella laboratory network has been performing very well and can support confirmation of cases and verification of measles elimination. However, the laboratory network still faces some challenges. The workload remains high and is even higher due to extensive fever and rash surveillance. Aside from the insufficient communication between EPI in China and laboratories in some provinces, there is also the difficulty of detecting and classifying each case as imported, import-related or endemic transmission. There is also insufficient financial support for laboratory equipment, supplies and reagents. Other challenges include timeliness of sample collection, transport of the throat swab specimens, and the need for quality assurance for the molecular detection in provincial and prefectural levels.

2.2.2b Jilin Province

Ms Jianhui Zhou of Jilin Provincial Center for Disease Control and Prevention talked about the epidemiology of measles and rubella, the vaccination programme and the measles laboratory network. The measles incidence in Jilin province was 0.8 per one million population in 2011 and 0.7 per one million population in 2012. In 2012, there were 21 measles cases in seven cities and 17 counties. There was a steady trend of low incidence of measles since 1986 except for two separate years, 2006 and 2009, during which there were measles epidemics. On the other hand, rubella incidence in Jilin province was under six per 100,000 population from 2004–2012 except for 2008, when incidence was 36.6 per 100,000 population. The reported routine immunization coverage rate of measles-containing vaccine is 98.2%. The 2010 coverage rate for supplemental immunization activity (SIA) for measles among children eight months to six years old was 98.3%. In 2012, the SIA for measles coverage rate was 97.8% among children eight months to three years old in some areas in Jilin province.

The laboratory has been using the Virion Serion IgM kits for measles and rubella. Siemens kit is not used. Proficiency test results from 2008 to 2012 were 100% for the prefecture measles laboratory network of Jilin province. A training session in 2011 was conducted for staff of the measles laboratory network of Jilin province. A score of 99% was obtained from the WHO on-site accreditation in 2008 and 2011. In 2010, there were 227 (67%) measles IgM positive from 339 measles samples. There were 18 (15.5%) measles IgM-positive in 2011 and 21 (19.8%) measles IgM-positive in 2012 (January to September). The laboratory was able to isolate 22 measles virus strains (H1) during 2008–2010. For 2011–2012, there was no genotype obtained. Using direct RT-PCR and restriction fragment length polymorphism (RFLP) for measles clinical specimen for genotyping, there was a total of 437 nasopharyngeal swab (NPS) samples tested from 2001 to 2012 and a total of 209 H1 genotypes identified. In 2010, out of 80 NPS samples tested by direct RT-PCR, 36 (15%) were confirmed measles positive and all 36 positive were H1 genotype by RFLP. Out of the 51 nasopharyngeal swab (NPS) samples tested in 2011, three
(5.9%) were confirmed measles positive and three were H1 genotype by RFLP. In 2012, out of 15 NPS samples tested, no measles positive and genotype were detected.

The laboratory was able to establish two methods of one-step RT-PCR for measles virus H1 genotype and vaccine strains identification. RT-PCR products are used for RFLP to identify H1 genotype of measles virus (567bp) and China vaccine strains of measles virus (438bp). The first method was applied in 20 laboratories, and about 186 measles virus strains from 20 provincial laboratories were identified using this method. The second method was used for industrial production in 2011 and all 31 provincial measles laboratories obtained rapid RT-PCR-RFLP kits for identifying China vaccine strains and wild strains. Phylogenetic tree showed that the most measles strains in 2010 and all the strains in 2009 are on one branch of the tree. Only three strains in 2010 are on a separate branch and exhibit 100% identities with a strain in 2005. As for rubella, there were also two established methods of one-step RT-PCR. One step RT-PCR was established both for diagnostic purposes (247bp) and for sequencing of rubella virus (988bp). Phylogenetic tree showed that rubella virus strains detected in Jilin province both in 2008 and 2012 belong to 1E genotype but were on different branches. The laboratory was also able to establish one-step RT-PCR for detecting and sequencing mumps virus. Phylogenetic tree showed that the mumps virus strains detected in Jilin province both in 2008 and 2012 belong to F genotype but were on different branches.

2.2.2c Yunnan

Ms Liqun Li, Chief Technologist from the Measles Laboratory at Yunnan Center for Disease Control and Prevention, presented on the measles and rubella epidemiology, running status of the provincial measles laboratory network and measles surveillance in the province of Yunnan. The measles vaccine was introduced in Yunnan in 1965. In 1983, the EPI was initiated and a two-dose vaccination strategy was adopted. Two SIAs, conducted in 2008 and 2010, resulted in decreased measles cases. Measles incidence was lowest in 2010 and has been maintained at a low level. As for rubella, epidemiological data showed that a rubella epidemic occurred in 2008. Compared to measles, the incidence of rubella has been higher since 2008. Consequently, it is necessary to control rubella during the measles elimination stage in China.

Yunnan provincial measles laboratory network was established in 2002 to include 16 prefectures and 129 counties. Since 2009, WHO PT is distributed to 16 prefecture laboratories, including 20 sera for measles and rubella and results were reported within 10 working days from receipt. All prefecture laboratories consistently obtained 100% scores, except for one prefecture laboratory in 2012. In addition, all 16 prefecture measles/rubella laboratories passed on-site accreditation with high scores. Training courses and workshops are conducted annually for the Yunnan provincial laboratory network since 2008. The in-house control was introduced in 2006 (based on the requirement of the national measles laboratory) and the Haitai kit (local kit) is used for both measles and rubella. The measles virological surveillance showed that since 2004, a total of 82 isolates were obtained with H1a (60 isolates), D11 (five isolates), or D9 (17 isolates). H1a has been continuously circulating in Yunnan province since 2004. D11 was first identified in the 2009 outbreak, imported from Myanmar, and detected in sporadic cases in 2010 and 2011. From January to September 2012, there were 12 H1a and 17 D9 that were isolated and imported D9 was found in the sporadic cases.

The measles laboratory at Yunnan introduced the molecular technique and the capacity of molecular detection has improved since 2009 when the imported genotype (D11) associated with the outbreak was found. Although Yunnan has made significant achievements, some challenges remain. Rubella virological surveillance also needs to be strengthened during the measles elimination. Yunnan province is bordered by Lao People's Democratic Republic, Myanmar and Viet Nam from where importation of viruses is a possibility, therefore, capacity for molecular
techniques needs to be maintained and strengthened. There is also lack of staff and turnaround of staff in the prefecture and county laboratories, affects the functioning of the laboratory and is a challenge.

2.2.3 Hong Kong (China)

Dr Wilina Lim from the Public Health Laboratory Centre (PHLC) in Hong Kong reported on the status of measles and rubella surveillance in Hong Kong (China). Measles became a notifiable disease in 1961 and in 1978, while congenital rubella syndrome was voluntarily reportable. Rubella became a notifiable disease in 1994, and congenital rubella syndrome was notifiable since 2008. There was an increase number of measles cases investigated from 2009 to 2012, correspondingly the number of samples tested also increased from 2009 to 2012, however, measles IgM positive cases decreased, from 10 positive measles cases in 2009 to three positive cases in 2012. There was no significant change in the number of rubella cases investigated since 2009 (1006 cases) to 2012 (978 cases). The percentage of rubella IgM positive was 1.5% in both 2009 and 2010, 3.3% in 2011 and 2.2% in 2012. In 2012, 37 rubella cases including three cases of congenital rubella syndrome were notified. There was a special vaccination campaign conducted in 1997, which included a schedule of MMR for those between one to 19 years old with history of zero or one dose prior to measles vaccination. This MMR schedule was included because data showed an increasing incidence among older children and adolescents (more than 51% of measles cases in 1997 were six to 19 years old). Among the measles cases in 1997, 50% had history of immunization (waning immunity or vaccine failure).

The national measles laboratory in Hong Kong (China) also uses in-house control (IHC) for measles and rubella IgM ELISA tests. The testing algorithm for rash illness was presented. Testing by IgM ELISA and PCR (on request) is performed for cases with samples collected within five days of rash onset. Other tests based on clinical information provided by clinicians are performed. It was also reported that measles and rubella virus were isolated from two and six cases, respectively in 2011 and 2012. Two mumps virus were also isolated in 2009 and 2011. The measles genotypes detected in 2011 and 2012 were D9 (five) and H1 (two), Rubella genotype detected were 1E (seven) and 2B (eight), for 2011 and same number in 2012. Mumps genotypes F, G, H were detected in 2011 and only F was detected in 2012. The laboratory received samples from other national measles laboratories of the WHO regional laboratory network for confirmatory IgM testing and genotyping. No samples or isolates for virus isolation were referred for confirmation. The laboratory has been performing RT-PCR and sequencing of complete measles genotyping window since January 2011 and established new genotyping RT-PCR for rubella in May 2012. The current challenge faced by the laboratory is developing the ability to evaluate real-time RT-PCR for rubella.

2.2.4 Japan

Dr Katsuhiro Komase from the National Institute of Infectious Diseases (NIID) discussed the current status of measles and rubella in Japan. Japan started measles vaccination in 1978 and introduced 2 dose schedule in 2006 and supplementary vaccination with MR vaccine in 2008. In 2008, a case-based surveillance for measles was introduced when from sentinel site reporting system, Japan switched to a case-based reporting system. The data showed an abrupt increase of reported cases in adults older than 15 years by about 200%–300% after the change in reporting. The Ministry of Health, Labor and Welfare issued a special guidance in 28 December 2007 related to the elimination of measles by 2012. A supplementary immunization campaign to last five years targeting children between 12 to 17 years old was implemented in 2008. Those between five and 22 years old will have an opportunity for MCV2 by 2012 under the new vaccination schedule. A significant decrease of 96% in the number of measles cases from 2008 to 2011 and further decrease of 1.6% from 2011 to 2012 were noted. There was a transition of
measles genotype detected from 2006 when only genotypes D5, H1 and A were detected, 80% of them were D5. In 2012, genotypes H1, D9, D4, D8 and A were detected and 70% was D8. The D5 genotype, which was dominant in 2006–2008, trailed out in 2009 and 2010 and was not detected in 2011 and 2012. Most of genotypes were considered to be imported cases or import-related cases, according to genetic information as well as epidemiological information in some cases.

The number of measles cases decreased from 11,007 in 2008 to 263 in 2012. It was noted that the ratio of laboratory-confirmed measles cases to reported measles cases also increased. While the use of PCR test was less than the use of IgM test in 2008–2010, the use of PCR assay was maximized in 2011 and 2012. The use of PCR or other tests to double check IgM-positive results may be useful to remove IgM false-positive case in measles elimination stage.

The number of rubella cases increased in 2011 (371) and 2012 (1,738), with males aged 20–50 years old comprising more than 70% of cases. Congenital rubella syndrome (CRS) cases were also reported in 2009 and 2011. Rubella virus genotypes obtained were 2B, 1j, and 1E. Sequences were slightly different even in the same genotype, suggesting that viruses may have been brought from multiple sources and/or may have been circulating silently in Japan for a long time.

2.2.5 Malaysia

Ms Janagi Naidu of the National Public Health Laboratory (NHPL) of Malaysia reported on the status of measles elimination, vaccination, surveillance and national laboratory data for 2009–2012. Upon initiation of the vaccination programme, the incidence of measles and rubella continued to decrease, most significantly during 1987–2009. Laboratory-confirmed measles cases were significantly higher in number compared to those clinically confirmed and epi-linked cases in 2011 and 2012. The number of cases reported increased following the success of a measles road show by zone conducted in November and December 2010 and January 2011. From 2010 (2/1,000,000) to 2012 (66.7/1,000,000), the number of reported cases significantly increased.

As part of quality assurance, the laboratory is using measles and rubella IHC in each test run and the 2012 results showed a mean of 0.229 within the +two standard deviation (2SD). In 2011, both measles and rubella showed a mean of 0.304 within the +three standard deviation (3SD). For both measles and rubella, confirmatory testing rates was 100% in 2010 and 2011 and 100 and 98% respectively in 2011. The PT results for 2010 was 100% for both measles and rubella and 95% and 100% respectively in 2011. The laboratory is also participating in the QA programme conducted by Royal College of Pathologists of Australasia (RCPA) and obtained a 100% score in 2012. The laboratory also performs virus isolation and molecular detection for measles and rubella. Testing algorithm for measles and rubella was also presented. There was a significant increase of samples tested from 2009 (2,269) to 2012 (4,486), with an increase in percentage of positive cases from 2% (2009) to 29% (2012). The rates of rubella positive cases significantly decreased to (13%) in 2010 from 42% in 2009, and have maintained at similar rates in 2011 and 2012. Measles virus isolation rate increased from 1% in 2009 to 11% in 2012. Rubella virus isolation had its peak rate in 2011 (9%). The measles virus genotypes obtained in 2012 were D8 (34) and D9 (44) while rubella virus genotypes were 2B (8) and 1E (1).

Malaysia designated a measles subnational laboratory in May 2011 to conduct serology testing for measles and rubella IgM for East Malaysia. The subnational laboratory is being monitored to ensure it adheres to the WHO standards. It also participated in the PT and obtained a score of 100% for both measles and rubella IgM. IHC is being used in every ELISA test run and has shown good results. Data from the subnational measles laboratory showed that measles
decreased from 55% positivity rate in 2011 to 7.9% positivity rate in 2012. However, rubella slightly increased from 7.8% positivity rate in 2011 to 10.6% positivity rate in 2012.

Case-based reporting through an online system, Sistem Maklumat Siasatan Measles (e-measles or SM2), was implemented. This online system enables the clinicians and staff at the district health office to notify any suspected case of measles within 48 hours. Specimens must be submitted along with a completed form that can be downloaded online. Results can also be checked by the District Health Office online. Subsequently, all suspected cases will be confirmed by the National Public Health Laboratory (NPHL) using ELISA method, virus isolation or PCR.

Major achievements were made in heightening awareness among clinicians and health officials in Malaysia regarding measles elimination, resulting in a higher number of cases being reported or notified. Reducing the number of underreported cases is a major step towards achieving the main objective of elimination programme. Furthermore, the increasing number of isolates and more genotypes deposited in MeaNS database is a positive sign that NPHL has strengthened its laboratory-based surveillance capacity. Some challenges encountered were inadequate clinical/epidemiological information for test interpretation and further testing, and some unsuccessful attempts to obtain second samples for equivocal cases.

2.2.6 Mongolia

Dr Nyamaa Gunregjav of the National Centre for Communicable Disease (NCCD) in Mongolia presented the history of measles elimination programme. Mongolia initiated the vaccination programme with MCV 1 in 1973, followed by MCV2 in 1986. Monovalent MCV was replaced by MMR in 2009 for children (vaccination schedule at 9 months and 2 years of age). Mandatory reporting of measles started in 1998. Case-based surveillance started in 2008 and the laboratory was accredited by WHO in 2004. After introduction of MCV2 in 1986, and high immunization coverage of over 95%, the confirmed measles case rate also showed downward trend. For the past three years since 2010 to 2012, there were no confirmed case of measles and the incidence rate has been zero since 2003. The last genotype, H1 (6), was observed in 2009. In terms of surveillance performance indicators, national reporting of discarded measles cases was 28.5% in 2012, higher than in 2011 (6.5%). It was noted that rubella positive cases increased from 5.1% (eight cases) in 2010 to 41.9% (233 cases) in 2012. In 2011, the rubella genotype obtained was 1E. The laboratory also performed mumps genotyping and the genotypes detected from 2009 to 2012 were H3 and F. The laboratory also participated in the WHO external QA programme, and the scores obtained from confirmatory and proficiency tests were 100% for both measles and rubella.

2.2.7 New Zealand

Mr Andrew Strathdee from Canterbury Health Laboratory (CHL) reported on the status of measles and rubella surveillance in New Zealand. The laboratory is using Biomerieux MiniVidas for measles IgG and rubella IgM/IgG, and Enzygnost for measles IgM ELISA testing. For PCR, measles real-time RT-PCR and rubella conventional RT-PCR are being used. Genotyping is also done using Qiagen RT-PCR kit. There were 136 samples tested for measles serology (IgM) in 2012 and eight (5.88%) were positive. In 2011, there were 718 cases tested for measles serology (IgM) and 175 (24.37%) were positive. In rubella cases, there were 40 samples tested for rubella IgM in 2012, of which, four (10%) were positive. In 2011, there were 85
samples tested for rubella IgM and four (4.70%) were positive. There were 114 measles cases in 2012 which were tested by RT-PCR, of which, 12 measles virus and one rubella virus were genotyped. D9 genotype was detected in 2011 while D4 was found in most of the measles cases in 2012. Current measles mumps rubella (MMR) immunization coverage rate is 91% among children two years of age, however, 95% coverage rate is needed to stop measles from spreading in the community. In 2011, there was an outbreak when the index case came back from the United Kingdom of Great Britain and Northern Ireland with measles and went to school while having the illness.

For the measles IgM confirmatory testing, there were good correlation of results between the laboratory and the RRL, 90% in 2011 and 100% in 2012. PCR results on measles also showed 100% correlation in 2011 (11/11) and 2012 (22/22).

2.2.8 The Philippines

Mr Rex Centeno from the Research Institute of Tropical Medicine (RITM) talked about the status of measles and rubella surveillance in the Philippines. While there was an increase in measles and rubella referrals during the first six months of 2011, the number has since been decreasing. After the second follow-up measles vaccination (modified door-to-door) for children between nine months to four years old in 2007, there has been increasing coverage of measles rubella (MR) vaccination. The trend was further strengthened by the 2011 MR-SIA activity (door-to-door) for children between the ages nine months to eight years.

There were 3807 people vaccinated for measles and 1107 for rubella in 2011. For the period of January to October 2012, there were 634 children provided with vaccines for measles and 112 for rubella. Confirmed measles cases were found in the age group of children less than eight years old both in 2011 and 2012. The majority of confirmed measles were found in Region 6, specifically in Negros Occidental, with almost 400 confirmed cases for the period of January to September 2012. For rubella, the majority were found in Region 7, specifically in Cebu. In 2010, there were excellent concordance rates, 98.7% for measles and 97.7% for rubella IgM, between the laboratory and the PHLC, Hong Kong (China). In 2011, there were 220 sera for retesting that showed excellent concordance rate for both measles and rubella IgM. The correlation of laboratory results was good in 2012 and the categorical discrepancies were minor, and not expected to affect patient investigation and management.

Based on molecular practice panel which was distributed at the regional WHO training course in November 2010, the quality of the sequences done by Hong Kong (China) laboratory was good. The phylogenetic tree sent by RITM were correct, the sequence was an exact match to the London 1i virus and the pictures of the detection and genotyping gels showed that the correct fragment sizes were obtained. For internal quality control, the laboratory implemented in-house controls for both measles and rubella IgM ELISA tests. Sequencing results of samples referred to Hong Kong (China) RRL for 2011 and 2012 obtained genotype D9 (11) for measles and three rubella genotypes, 1j (two) and 2B (one).

Recent achievements include issuance of a measles and polio quarterly bulletin, and regular participation in vaccine-preventable diseases (VPDs) meetings with the Department of Health and regional surveillance officers to advocate support for measles laboratory. There have also been training sessions held for regional officers to learn about alternative methods for diagnosis and provision of dedicated shipment boxes for VPD surveillance. The renovation of the molecular laboratory for the virology department was also completed. One of the challenges that the laboratory is facing is the failure of some regions with high incidence of measles to collect samples for virus isolation. In addition, there is also some difficulty in distinguishing
measles cases from diseases with overlapping symptoms as measles. There is also presence of mycoplasma contamination in Vero/hSLAM cell line.

2.2.9 Republic of Korea

Mr Seung Tae Kim from the National Institute of Health (Korea CDC) presented the status of MMR vaccination and national laboratory data with testing quality control and external quality assurance. Among Korean children, the vaccination rate for two-dose MMR has reached more than 95% before entry in primary school. In 2012, it was confirmed that the incidence rate of measles cases is less than one per one million population. The number of measles confirmed cases decreased from 2010 to 2012 with 43 positive cases by RT-PCR in 2010 and 15 positive cases in 2011. All 43 positive cases from 2010 were genotyped and the results all showed H1 strain. Also, all 15 positive cases from 2011 were genotyped and found to be D9 strain except for four vaccine-related cases. The phylogenetic tree of measles virus showed that the 2010 measles outbreak in Incheon was caused by H1 strain, while the 2011 measles outbreak in Gyeongnam was caused by D9 strain. In 2012, among 12 reported cases of measles cases, only four were laboratory confirmed, while all of the 27 reported rubella cases were not confirmed by the laboratory. The measles IgM positive cases comprised 17% of entire rash-febrile illness and over 39% of human herpesvirus-6 or parvovirus-B19 IgM positive cases were differentially diagnosed.

A robust QA programme is being implemented in the laboratory. The IHC results for measles and rubella were good. In the confirmatory testing, the accuracy of measles and rubella IgM detection was more than 90% for 2010–2012. The molecular proficiency test results in 2010 showed 100% for both measles and rubella.

The laboratory is also performing analysis of mumps virus. The phylogenetic tree of mumps virus showed that H and I strains were detected in 2010 and 2011, while only I strain was detected in 2012. Evaluation of results in 2012 external quality assessment showed 10 PHERIs and four private diagnostic centres had reported final results within 35 days after distribution and most of the participants had returned good results. Significant achievements of the programme are the low incidence rate of confirmed measles cases in 2012, which is less than one case per million, Korean mumps virus isolate was registered in WHO reference strain and 17 PHERIs can confirm measles and rubella laboratory diagnosis. The challenges are the lack of staff members in measles/rubella laboratory and the launch of MMR real-time RT-PCR system.

2.2.10 Singapore

Dr Lui Sook Yin of National Measles Laboratory at Singapore General Hospital (SGH) started the report by discussing the history and current status of measles vaccination programme in Singapore. Measles vaccination began in 1976 and was made compulsory by law in 1985 for children aged one to two years old. In January 1990, monovalent measles vaccine was replaced by trivalent MMR. The two-dose MMR vaccination was implemented in January 1998 and also served as replacement for monovalent rubella. In late 2011, it was recommended that the first dose of MMR should be given to children at 12 months of age and the second dose will be given at 15 to 18 months of age.

The SGH is using Siemens Enzygnost kits both for measles and rubella IgM. SGH also uses immunofluorescence for measles antigen identification and Vero/hSLAM for virus isolation. The laboratory performs laboratory testing of measles and rubella on samples referred by clinicians and also through an enhanced measles surveillance programme wherein clinically-confirmed measles are referred for laboratory confirmation. Quality assurance is characterized by strong implementation of some measures such as quality control, equipment monitoring and
maintenance. Furthermore, technical staff are trained and competency assessed before they are allowed to perform laboratory activities. Aside from being accredited as a WHO measles and rubella national laboratory, the laboratory has also been accredited by the College of American Pathologists (CAP) and by the Joint Commissions International (JCI). The laboratory also participates in different PT programmes conducted by College of American Pathologist (CAP), Royal College of Pathologists of Australsia (RCPA), WHO and US CDC.

Confirmatory samples are sent to the Hong Kong (China) RRL and results of confirmatory testing from the last three years showed good concordance rates both for measles IgM (93%–100%) and rubella IgM (100%). The laboratory obtained a 100% score in the WHO proficiency testing for measles and rubella. From 2010 up to September 2012, a total of 628 cases were tested for measles IgM, while 1468 cases were tested for rubella IgM. Among the measles cases tested, there were a total of 86 (13.69%) positive cases. Sixty (4%) positive were found among the rubella cases tested. There were 16 (7.3%) measles isolates in 2010, 42 (15.4%) in 2011, and four (4.1%) isolates in 2012. The total number of measles virus sequenced from 2010–2012 was 66. In 2010, three measles genotypes were detected, H1 (one), G3 (2) and the dominant genotype was D9 (13). In 2011, there were four genotypes obtained, D9 (33), D8 (11), D4 (1), and G3 (1). Only D9 (4) genotype so far has been obtained in 2012. A pilot project was conducted for rubella genotyping between July 2011 and March 2012. Forty-five samples for measles antigen IF confirmation and seven rubella IgM equivocal/negative samples were processed for virus isolation and confirmed by IF using anti-rubella monoclonal antibodies. There were four rubella virus isolated and these were subsequently genotyped. The rubella virus genotypes obtained were 1E (two) and 2B (two) from September 2011 to January 2012.

With regard to laboratory data reporting, the Ministry of Health is notified of all positive measles and rubella IgM cases. Monthly data are reported to WHO and the Ministry of Health. Measles genotyping results are updated on the WHO measles genotype database within two months of receipt of specimens. The remaining challenges for the laboratory are: (1) the lack of monitoring of cases confirmed by laboratories other than the National measles laboratory (2) lack of better coordination between laboratory and EPI.

2.2.11 Viet Nam (Hanoi)

Ms Trieu Thi Thanh Van from the National Institute of Hygiene and Epidemiology (NIHE) presented the current status of the national measles immunization programme and the measles and rubella surveillance in Northern Viet Nam. Routine immunization for MCV1 for children aged 9 months started in 1981. MCV2 into routine immunization started in 2006 covering children six years of age until 2010. Since 2011, MCV 2 in given at 18 months of age. In 2010, there were 63 (100%) provinces which conducted SIAs for children one to five years old. The plan for 2013–2014 is to administer MR vaccine for children aged nine months to 14 years old. Because of vaccine shortage in 2007, there was a decrease of coverage of MCV1. However, the lowest incidence rate of measles obtained in 2007 was 0.2 per 1 000 000 population. There was a significant increase of incidence (91.1 per 1 000 000 population) in 2009 but decreased to 29.8 per 1000 000 population in 2010 and 8.6 per 1 000 000 population in 2011. In 2010, 278 (28%) measles IgM samples were tested positive, while in 2011, seven out of 1433 (0.5%) samples were positive. In 2012, there were four measles IgM positive (1%) out of 412 measles cases. For rubella IgM testing, there were 501 (44%) out of 1130 which tested positive in 2010, 1399 (57%) in 2011, and 93 (23%) in 2012.

Quality assurance measures include the use of IHC, participation in WHO PT and confirmatory testing by RRL. The laboratory has been receiving and using Siemens measles and rubella kits from WHO since 2009. The proficiency test scores of 100% were achieved for both
measles and rubella from 2006 up to 2012. The laboratory obtained 100% for 2011–2012 in the
confirmatory test for measles.

For rubella virus samples, confirmatory test scores were 84%–100% for 2010-2012. Virus
isolation is done using throat swab (TS) samples from 2006 and only samples showing
cytopathic effect (CPE) are tested for RT-PCR. However, from 2010 to 2011, all samples for
virus isolation were also tested by RT-PCR. In 2010, 25 (89.3%) measles viruses were detected
by RT-PCR out of 28 TS samples, while only three (10.7%) measles viruses isolated. In 2011,
24 rubella viruses were detected by RT-PCR from 55 TS samples, while no virus isolated. The
data showed that RT-PCR is more sensitive than virus isolation in detecting measles and rubella
viruses. However, virus isolation has to be performed because characterization of measles and
rubella isolates are very important in the elimination phase. Genotyping data showed that only
H1 measles strain and 2B rubella strains have been circulating in Viet Nam since 2006. There
were 11 measles viruses sequenced in 2010 and six rubella viruses sequenced in 2011 in NIHE.
The genotype obtained for measles virus in 2010 was H1 and in 2011, 2B genotype was obtained
for rubella virus. PHLC in Hong Kong (China) has also performed genotyping on samples
referred by NIHE and data has shown that only H1 measles virus strain and 2B rubella virus
strain are circulating in Viet Nam. PHLC obtained seven H1 measles virus genotypes and two
2B rubella virus genotypes in 2010 and one H1 measles virus genotype and three 2B rubella
virus genotypes in 2011.

There are three congenital rubella syndrome (CRS) surveillance sites in Viet Nam which
are the National Hospital of Pediatrics in North Vietnam (Hanoi), the Children’s Hospital No.1
and Children’s Hospital No. 2 in South Viet Nam (Ho Chi Minh City). The laboratory was able
to confirm from CRS surveillance 168 positive for rubella IgM (84%) out of 199 samples tested.
Majority of the positive cases (37 cases) came from the Hanoi area.

2.2.12 Viet Nam (Ho Chi Minh)

Ms Thanh Giang Dang of Pasteur Institute presented on the measles and rubella
surveillance in Southern Viet Nam. The national measles vaccination programme started in 1981
for children from nine to 11 months old, with a second dose at the age of six years old. However,
the schedule of the second dose of vaccination was modified in 2010 to be administered to
children at 18 months of age. Since 2010, the laboratory has been accredited as a WHO measles
and rubella national laboratory. It performs virus isolation and identification of measles and
rubella viruses from 20 provinces. The laboratory has also participated in the new surveillance
for CRS since 2011. The laboratory has also the capacity to perform PT-PCR and sequencing.

The laboratory used ELISA kit provided by WHO (Siemens) and Pasteur HCM (Serion) in
2011. In 2012, all ELISA kits were provided by WHO. Out of 4250 measles samples tested in
2010, there were 885 (20.82%) positive for measles IgM. In 2011 and 2012, there were only six
(less than 1%) positive out of 1467 samples and one (less than 1%) out of 259 samples,
respectively. This data showed that measles prevalence is decreasing. For rubella samples, there
were 1834 (46.6%) positive for rubella IgM out of 3933 in 2010 and 1077 (62.9%) positive out
of 1713 samples in 2011. In addition, 23 (8.9%) of 258 samples in 2012 were rubella IgM
positive. It was noted that majority of measles IgM positive occurred in the months of January
2011 and July 2012. For the rubella IgM, March 2011 and February 2012 had the highest
numbers of positive cases. The timeliness of reporting within seven days improved from 24% in
2011 to 97% in 2012. Proficiency test scores of 100% were obtained in 2010 and 2011 for both
measles and rubella IgM. The laboratory was ISO 15189 accredited in 24 January 2011. A
robust QA programme is being implemented in the laboratory.
The total rubella IgM positive in 2011 for CRS surveillance in two hospital sites was 110 (82.7%) out of 133 samples, whereas, total IgG positive were 13 (59%) out of 22 tested. For the period of January to September 2012, the total rubella IgM positive for CRS was 77 (48.4%) out of 159, while total IgG positive was 45 (54.8%) of 82 tested. The laboratory was able to obtain two measles virus positive and two RT-PCR rubella virus positive in 2010. Genotyping results showed that the circulating measles virus strain is H1 and rubella virus strain is 2B.

Some of the challenges are the need to train the provinces on specimen collection including throat swabs since receiving TS samples is rare, and the need for the protocol of nested RT-PCR since it is difficult to do RT-PCR for serum.

2.3 Practical sessions

The hands-on training session was conducted in the laboratory of the Virology Division, PHLC, Centre for Health Protection, Hong Kong (China). It included five days of practical sessions. Participants worked in six groups, and different rooms were used for different steps.

(1) Group A: Australia, New Zealand

(2) Group B: China

(3) Group C: Malaysia, Mongolia

(4) Group D: Philippines

(5) Group E: Singapore, Republic of Korea

(6) Group F: Viet Nam

The first day of practical sessions on 29 October 2012 included sessions one and two on RNA extraction from FTA cards containing cell lysates for measles and rubella, and setting up real-time RT-PCR for measles. The practice panel of FTA filter paper discs and the measles real-time RT-PCR kit were distributed by US CDC for the measles/rubella laboratories in the WHO laboratory network.

The second day of practical sessions included session three on setting up real-time PCR for rubella and introduction to ABI 7500. Also, the group performed analysis of measles and rubella real-time PCR. Session four, which ran through the night, was on setting up genotyping RT-PCR for measles and rubella.

The third day of practical sessions included session five on analysis of genotyping RT-PCR by agarose gel electrophoresis followed by session six on cleaning up of PCR products and template gel. Sessions seven and eight were on setting up sequencing reactions for measles and rubella and cleaning up sequencing reactions.

The fourth day of practical sessions included session nine on preparing samples for loading to sequencer using ABI 3730. The sequence analysis using mega was introduced. There was a demonstration of measles and rubella sequence alignments. The participants were asked to analyse data from real-time or genotyping PCR and practice with chromatogram files supplied by US CDC.
The fifth day of practical sessions included data reporting and sequence analysis. The participants were asked to practice sequence analysis and analysis of sequence data generated from the fourth day experiment. A post-assessment quiz was given to all participants.

On the last day, 3 November 2012, the results of the quiz were reviewed. Participants also provided an assessment and feedback on the WHO training course. The practice samples for molecular PT for measles and rubella were distributed to the participants.

3. CONCLUSIONS

3.1 General

During the five and a half days of intensive hands-on practical sessions, lectures, and group work, the main objectives of the workshop were fully achieved. At the end of the workshop, the knowledge and skills of the participants were enhanced. They were able to understand, explore and perform molecular detection of measles and rubella viruses using the new real-time PCR and sequencing and sequence data analysis of measles and rubella. The participants were further familiarized with laboratory quality assurance of molecular detection of measles and rubella virus.

3.2 Workshop evaluation

The participants’ feedback confirmed that the workshop met its objectives. Administrative arrangements by PHLC were excellent. All participants were allocated a locker during the entire workshop and clear instructions to the venue for each session were provided by PHLC staff.

Participants were encouraged to contact and collaborate with each other, the facilitators and WHO Regional Office for the Western Pacific to follow up on practical issues such as quality assurance (QA). QA includes reporting of molecular PT results, in-house control samples, confirmatory testing, genotype data management and reporting, and strengthening molecular detection capacities in each network laboratory.

The training ran smoothly throughout the practical and lecture sessions due to the full support from PHLC staff. The participants efficiently completed all the practical sessions and understood issues addressed during the training. The training schedule provided adequate time in performing practical procedures at a reasonable pace and sharing of information among the participants. The duration of each presentation also allowed ample time for further discussion on theoretical and technical issues.

3.3 Outcomes of training

All participants were familiarized with the molecular detection of measles and rubella viruses by RT-PCR, enhanced sequencing capacity and laboratory quality assurance of measles and rubella diagnosis. They were also informed of the requirements for reporting genotype and sequence data to WHO and MeaNS.

At the end of the workshop, the WHO practice molecular measles and rubella PT panel and reagents were distributed to participants.
3.4 Follow-up to the workshop

Participants were requested to complete PT and report results within six weeks to US CDC and WHO laboratory coordinator using standardized reporting format. Most of the participants provided the PT results within the agreed time. US CDC provided comprehensive feedback to the countries on their PT results.