Training Workshop Report

The 4th Regional Hands-on Training Workshop on the Laboratory Diagnosis of Measles and Rubella focusing on Molecular Diagnosis

Hong Kong (China)
22–27 November 2010
REPORT
THE 4TH REGIONAL HANDS-ON TRAINING WORKSHOP ON THE LABORATORY DIAGNOSIS OF MEASLES AND RUBELLA FOCUSING ON MOLECULAR DIAGNOSIS

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NOTE

The views expressed in this report are those of the participants of the 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 Hands-on Training on the Laboratory Diagnosis of Measles and Rubella, which was held in Hong Kong (China) from 22 to 27 November 2010.
The Hands-on Training on the Laboratory Diagnosis of Measles and Rubella in the Western Pacific Region was held at the Public Health Laboratory Centre (PHLC) in Hong Kong (China) from 22 to 27 November 2010.

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 the national measles/rubella laboratories of China, Japan, Malaysia, Mongolia, New Zealand, the Philippines, the Republic of Korea, Singapore and Viet Nam. In addition to support from the WHO Secretariat, the training was facilitated by temporary advisers from the United States Centers for Disease Control and Prevention (US CDC), Chinese Centers for Disease Control and Prevention (China CDC), and the National Institute of Infectious Diseases (NIID), Japan.

The objectives of the workshop were:

(1) to enhance knowledge and skills of national measles and rubella laboratory staff in:
   (a) molecular detection of measles and rubella viruses (RT-PCR and sequencing);
   (b) laboratory quality assurance of measles and rubella diagnosis; and

(2) to discuss regional data management using the new laboratory reporting format.

The hands-on training, which consisted of lectures, country reports and practical sessions, focused on understanding the needs and role of the measles/rubella laboratory network and learning and training in the use of IgM assay kits.

Overall, the participants were positive in their feedback and they considered the workshop to have met its objectives and the schedule and administrative arrangements to be well organized.

The workshop participants were encouraged to contact each other and the facilitators after the workshop to maintain the success of the measles/rubella laboratory network.
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Keywords:

Measles-diagnosis/ Rubella-diagnosis/ Laboratory personnel-training/ Laboratory techniques and procedures/ molecular diagnosis/ sequencing
1. INTRODUCTION

The Region established a measles elimination goal in 2003 and set 2012 as the target year for elimination in 2005. Since then, the Regional Measles and Rubella Laboratory Network, consisting of one global specialized laboratory (GSL) and three regional reference laboratories (RRLs), has been developed, with 16 national and 362 subnational laboratories in China. The network plays a critical role in monitoring the progress of measles elimination and rubella control. It does this by confirming measles and rubella cases and providing genotyping or molecular data to understand the epidemiology of measles better. Timeliness in providing reliable laboratory data is critical to identify and respond to imported or endemic chains of measles transmission, particularly as countries approach measles elimination.

Following the Global Poliomyelitis LabNet model, a WHO accreditation system for measles and rubella laboratories was developed at the global level to ensure the performance quality of network laboratories. As quality assurance measures, WHO proficiency testing and confirmatory testing programmes have been established. All network laboratories in the Region send a proportion of serum samples to network RRLs or GSLs that are accredited annually based on WHO accreditation criteria.

Detection of measles or rubella immunoglobulin (IgM) in serum is the standard test for laboratory diagnosis of measles and rubella. There are several commercial IgM enzyme-linked immunosorbent assay (ELISA) kits available, many of which are used among WHO network laboratories. WHO has recommended the use of Siemens IgM kits for measles and rubella among WHO Global Network Laboratories, but other kits are also used in this and other WHO Regions.

As the role of the Measles and Rubella Laboratory Network also extends to molecular surveillance, laboratories with the capacity for virus isolation, molecular diagnosis and sequencing are encouraged to perform those additional functions. 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 sequencing information are submitted to the WHO genotype and MeaNS database by national or regional laboratories. Genotype data on recent measles virus strains are available from most countries, except Pacific island countries (PICs).

The Hong Kong (China) Measles/Rubella Laboratory was designated as a WHO RRL in 2007. This was to provide additional Regional support for confirming samples from other national laboratories and genotyping of measles and rubella viruses circulating in the Region. In 2009–2010, the Hong Kong (China) RRL provided excellent support in identifying genotypes of measles viruses circulating in Viet Nam, Cambodia, the Lao People's Democratic Republic, Mongolia, Macao, the Philippines and Malaysia using confirmatory serum or virus isolation samples.

To strengthen the capacities of network laboratories for measles and rubella virus isolation and identification and to discuss the progress and challenges of the network, laboratory staff were invited for regional meetings or hands-on training workshops.
To improve country reporting of measles laboratory data to WHO’s Regional Office for the Western Pacific, a new case-based reporting format was developed. This has been in use since January 2008. By the end of 2010, 15 out of 17 laboratories that were supposed to send monthly laboratory data to WHO’s Western Pacific Regional Office were sharing data on a monthly basis. Laboratory data including measles genotypes identified from each country have been included in WHO’s Western Pacific Regional Office Measles and rubella bulletin since 2011.

In 2009, the Third Regional Hands-on Training on the Laboratory Diagnosis of Measles and Rubella was organized at the Hong Kong RRL. This aimed to enhance laboratory capacities in priority countries in the Region. Participants included laboratory staff from Cambodia, China, Fiji, the Lao People’s Democratic Republic, Malaysia, Mongolia, the Philippines and Viet Nam. This 5-day course covered ELISA techniques using serum and dried blood spot (DBS) samples, virus isolation and molecular detection for measles and rubella.

The Fourth Laboratory Hands-on Training workshop focusing on molecular detection of measles and rubella viruses was organized for 5.5 days. It aimed to enhance the genotyping capacity of national measles/rubella laboratories in the Region as a follow-up to the Third Hands-on Training. Network laboratories that could benefit from training on molecular detection and analysis were to be invited. At the end of the workshop, participants were provided with the Annual WHO Measles/Rubella Proficiency Panel samples prepared by the Victorian Infectious Diseases Reference Laboratory (VIDRL), Australia. All laboratories were requested to test these samples and report results to WHO’s Western Pacific Regional Office within 14 days of the samples being received.

1.1 Objectives

(1) To enhance the knowledge and skills of national measles and rubella laboratory staff in:

(a) molecular detection of measles and rubella viruses by reverse transcription polymerase chain reaction (RT-PCR) and sequencing; and

(b) laboratory quality assurance of measles and rubella diagnosis.

(2) To discuss Regional management using the new laboratory reporting format.

1.2 Participants

The training session was attended by 13 participants from WHO-designated national measles/rubella laboratories in China (3), Japan (1), Malaysia (1), Mongolia (1), New Zealand (1), Philippines (2), Singapore (1), the Republic of Korea (1) and Viet Nam (Hanoi [1], Ho Chi Minh [1]). In addition to the WHO Secretariat, temporary advisers from the United States Centers for Disease Control and Prevention (US CDC), National Institute of Infectious Disease (NIID) Japan and Public Health Laboratory Centre (PHLC) Hong Kong attended as facilitators. A list of participants is included in Annex 1.
1.3 Opening remarks

Dr Wilina Lim, consultant and medical microbiologist at PHLC Hong Kong (China) welcomed the participants, opening the hands-on training workshop with an introductory speech.

2. PROCEEDINGS

2.1 Lecture sessions

2.1.1 Update on Western Pacific Regional Office Measles and Rubella Laboratory Network

Dr Youngmee Jee gave a presentation on Regional progress of measles elimination, confirming that the number of measles cases in 2010 had dropped 58% in the Western Pacific Region. Measles incidence, immunization coverage, supplementary immunization activities (SIAs) and challenges to achieving measles elimination by 2012 were also presented. Updates on Regional Measles and Rubella Laboratory Network performances were provided. All GSLs and RRLs, 10/13 national labs – including Fiji but excluding three PIC labs, plus all 31 provincial labs in China – were accredited as of 22 November 2010. Results of confirmatory testing were presented and concordance rates of most laboratories were >90% in 2010. Ways of strengthening the quality of the performances of Measles/Rubella Laboratory Networks and enhancing the Regional capacity for virus isolation and genotyping were discussed. Recent measles outbreaks in the Region, including Viet Nam, the Philippines and New Zealand and increased measles activity in Cambodia, were discussed with country participants. Recommendations from the Second Laboratory Network meeting were reviewed and objectives of the training were introduced.

2.1.2 Importance of molecular epidemiology and timely reporting of genotype information

Mr David Featherstone presented the importance of molecular surveillance and how it can be used for identifying geographical origin and tracking transmission pathways of viruses. Indicators for determining measles elimination, variables of genotype/sequence database and current status of genotype and sequence database were discussed. Key messages were: (1) The timely reporting of information on sequences is critical to monitor the programme and determine elimination. (2) It is necessary to identify sequences from all outbreaks and chains of infection. (3) Timeliness of genetic information reporting will be monitored through the accreditation check-list. (4) Sequences of measles viruses can be recorded on the MeaNS database, while genotype data can be recorded on the WHO HQ database.

2.1.3 Molecular technique for measles virus detection/genotyping by RT and real-time PCR

Dr Paul Rota introduced a molecular technique for measles virus detection. He pointed out the importance, lessons learnt and limitations in virologic surveillance and virus detection. The algorithm for molecular testing and the use of conventional or real-time PCR for case classification (vaccine reaction, primary and secondary vaccine failure), molecular surveillance and how to interpret real-time RT-PCR results were explained. Reference strains of measles viruses and examples of measles virus surveillance in the United States of America were presented. A full explanation of measles genotyping kits prepared by US CDC and TaqMan real-time PCR and interpretation of results was given. Possible shipping of vial isolates on filter
papers and stability of measles RNA on fluorescent treponemal antibody (FTA) cards for one month were shown. The FTA cards could possibly make sample transportation between laboratories easy.

2.1.4 Molecular techniques for rubella virus: diagnostic and genotyping reverse transcription (RT) and real-time PCR

Dr Joseph Icenogle introduced diagnostic and genotyping RT-PCR methods for rubella, and some challenges of direct sequencing of rubella virus from the sample. These were due to: (1) high guanine-cytosine (GC) content, (2) amplicon/sequence window size, (3) primer design, and (4) copy number in clinical samples. Currently available rubella genotypes from the countries concerned were presented. Genotypes 1E, 1G and 2B rubella strains are widely distributed. In this Region, genotypes 1E, 2B and 1j were detected in 2009–2010.

2.2 Country reports

2.2.1 China

Dr Xu Song Tao from the Chinese Centers for Disease Control and Prevention (China CDC) presented the epidemiology of measles and rubella laboratory testing and quality control, virus surveillance and imported measles cases in China. The incidence of reported measles cases in 2009–2010 (January–October) was the lowest since the establishment of the reporting system. The monthly and age analysis of measles-positive cases shows April peaks and the highest incidence among infants under one year old. Nationwide SIAs were conducted from 11–20 September 2010. The aim was to increase rapidly the immunity level of the target population of more than 100 million, to prevent the spread of the virus and reduce its incidence.

More than 80% of samples from sporadic cases and more than 95% of outbreak cases were laboratory-confirmed from January 2009 to May 2010. The Measles Laboratory Network in China tested 314 outbreak cases in 2009, and 293 were confirmed. From January to May 2010, 143 outbreaks were reported and 137 were laboratory-confirmed. Among 48,107 suspected sporadic cases in 2009, samples were collected from 39,459 cases, and 23,090 were confirmed as measles IgM-positive. Among 29,058 suspected sporadic cases from January to May 2010, samples were collected from 18,217 cases, and 12,323 were positive for IgM. For quality control, annual confirmatory testing, proficiency testing and on-site review for WHO accreditation are conducted. Each year, 10–13 provincial laboratories are assessed using the WHO measles/rubella laboratory check-list. Measles/Rubella IgM Proficiency Testing samples for 31 provincial laboratories were sent out by VIDRL, Australia. The results from 31 provincial labs were collected by China CDC and later sent to WHO and VIDRL. All provincial laboratories obtained excellent scores. On-site reviews of provincial laboratories were conducted by a WHO accreditation team and RRL. Twelve provincial labs passed on-site review/ accreditation in August 2010. The Tibet CDC Measles Laboratory passed an on-site review for the first time since WHO accreditation was initiated.

The incidence of reported rubella cases increased from 2004 to 2008, but decreased in 2009. Most rubella outbreaks occurred in primary or middle schools, and more outbreaks were detected in rural schools than in cities. In terms of age distribution, the population of 5-10 year-olds had the highest incidence of measles. In 2010, from January to October, out of 40,762 reported cases, 6,459 were laboratory-confirmed and the incidence rate was 3.07/100 000. In 2009, 118 rubella outbreaks were reported and 106 were laboratory-confirmed. In 2010 (from January to May), 25 outbreaks were reported and 24 were laboratory-confirmed. In addition, 25,877 sporadic rubella cases were reported in 2009; samples were collected from 8,931 cases,
and 3148 were laboratory-confirmed. In 2010 (from January to May), 5723 suspected cases were reported; samples were collected from 2481 cases, and 1081 were laboratory-confirmed. In 2009 and 2010 (January–May), >89% and around 96% of rubella outbreaks respectively were laboratory-confirmed. From suspected rubella outbreak cases, 867 and 89 cases were confirmed as measles in 2009 and 2010 (January–May) respectively. A three-year WHO–Ministry of Health collaborative project on rubella and congenital rubella syndrome (CRS) surveillance was initiated in 2010.

Many provincial laboratories as well as China CDC isolated measles and rubella viruses. From January–October 2010, 227 measles and 28 rubella virus strains were isolated. Measles isolates and genotyping results for 2010 were presented. In 2010, 224 H1, one D9 and one D11 strains were detected from January to October. From 1999 to October 2010, 208 rubella strains were identified, with the 1E genotype being the predominant strain, representing 85%. Rubella genotype 1E strains were isolated from 19 provinces throughout this period, while 2B strains were only detected in 2008. China CDC has trained provincial laboratories in RT-PCR, real-time PCR and RT-PCR-restriction fragment length polymorphism (RFLP). Challenges for China CDC include the integration of EPI data with laboratory data, improving collection of virus isolation samples from all chains of transmission and timeliness of shipping virus isolates from provinces. Some provincial laboratories that can sequence virus isolates by themselves also need to share their sequence results with China CDC and ship virus isolates to China CDC on a monthly basis for confirmation.

2.2.2 Japan

Dr Nakatsu Yuichiro from the National Institute of Infectious Diseases, Japan presented a measles vaccination schedule and confirmed the introduction of a newly revised reporting system for measles from 2008 and the 5-year SIA targeting teens from 2008 to 2012. Measles control strategies in Japan have included a 2-dose measles-containing vaccine (MCV) schedule. The second dose (MCV2) has been administered at age 5–6 years since 2006, in accordance with the recommended WHO Western Pacific Regional Office measles elimination strategy. With the introduction of measles SIAs from 2008, measles cases in 2009 and 2010 decreased to 741 in 2009 and around 400 in 2010, compared with 11 015 cases in 2008. The number of reported measles cases is decreasing and may drop to fewer than 500 cases/year in 2010 (<4.0/million). The reduction in measles cases in 2009 and 2010 is probably linked to the measles epidemic in 2007 and 2008 as well as to the new vaccination strategy started in 2008. The proportion of laboratory-diagnosed measles cases has gradually been increasing, from 38.2% in 2008 to 71.7% in 2010, with a dramatic national decrease in measles cases. While commercial laboratories where most measles testing is conducted are using IgM ELISA (Denka Seiken kit), public health laboratories are using RT-PCR. The problems in measles surveillance in Japan include: (1) sending samples to public health labs rather than commercial labs, and (2) differentiation of possible measles IgM false-positive cases when measles cases are deceeding, suggesting several tests should be conducted to identify false-positive cases.

2.2.3 Malaysia

Ms Wan Noraini Wan Yussof from the National Public Health Laboratory (NPHL) Malaysia presented the measles vaccination programme, national vaccination coverage and laboratory testing of measles and rubella conducted at NPHL. A vaccination schedule with single measles vaccine at six months, followed by two doses of measles/mumps/rubella (MMR) vaccine at 12 months and seven years, is used in Sabah state. Other areas have a two-dose vaccination schedule at 12 months and seven years. Reported vaccination coverage in 2008–2009 was 95%.
Three laboratories at NPHL – the Serology Lab, Virus Isolation Lab and Molecular Lab – are involved in measles serology and virus identification. Out of 2223 and 1156 suspected measles cases in 2009 and 2010, 51 cases were laboratory-confirmed in 2009 and 51 in 2010. In 2009, most cases were from Selangor and Kuala Lumpur, and in 2010 most were from Kedah and Selangor. Cases were mainly in children under seven years and the age group under one year showed the highest incidence in 2010.

NPHL uses multiple testing algorithms for the laboratory diagnosis of measles/rubella for diverse epidemic situations. This laboratory tested 1176 samples in 2007, 3046 samples in 2008 and 2269 in 2009 for measles. It detected 44 measles-positive in 2007 (3.7%), 90 positive in 2008 (3%) and 56 positive in 2009 (2.5%) respectively. Nine hundred and forty-three samples in 2007, 2068 samples in 2008 and 3184 in 2009 were tested for rubella. Out of those, 468 samples were positive in 2007 (49.6%), 851 were positive in 2008 (41.2%) and 730 were positive in 2009 (13.6%) respectively. During 2007–2010, nine samples were genotyped and D9 strains (n = 6) were detected in 2008, while G3 strains were detected in 2009 (n = 1) and 2010 (n = 2).

Confirmatory samples were sent to the RRL in Hong Kong in 2010, and good correlation was shown between the results from the two laboratories. Remaining challenges include collecting early virus isolation samples for genotyping. A new measles surveillance guideline is being developed.

2.2.4 Mongolia

Dr Rentsen Tuul from the National Center for Communicable Diseases in Mongolia gave a presentation on the laboratory testing algorithm and the results of IgM testing for measles and rubella from 2007 to 2010. The National Measles Laboratory uses standard enzyme immunoassay (EIA) techniques and is strengthening virus isolation and molecular detection capacities for measles and rubella. In 2007, 2030 samples were tested for measles and rubella. Only 13 were positive for measles (0.6%), while 964 were positive for rubella (47.4%). In 2008, 301 samples were tested for measles and 30 of these were positive (9.9%); 67 samples out of 279 tested for rubella were positive (24%). In 2009 and 2010 (January–October), 177 and 130 samples were tested for measles respectively. Only three were measles-positive and 11 were rubella-positive.

2.2.5 New Zealand

Mr Kevin Barratt from the Canterbury Health Laboratories in New Zealand presented laboratory testing results for measles and rubella, including serology and RT-PCR results in 2010. This laboratory uses Siemens kits for measles IgM, but uses Biomerieux for rubella IgM, measles IgG and rubella IgG. Real-time PCR and virus isolation are also performed using throat swabs, urine, blood cells and serum. In 2010, 133 and 57 samples were tested for measles and rubella respectively. Five were measles-positive, six were measles-equivocal and one was rubella-positive. A total of 129 samples were tested using RT-PCR and 14 samples were positive for measles. Among those RT-PCR positives, D8 genotype strains were detected in March, April and August 2010. In Auckland, 32 measles cases were reported and five were laboratory-confirmed by LabPLUS Auckland. All cases were from a non-vaccinated family and an index case was a child returned from India. This laboratory receives external quality assurance panel samples as well as WHO Proficiency Panel samples.
2.2.6 The Philippines

Mr Rex Centeno from the Research Institute of Tropical Medicine (RITM) presented the results of laboratory diagnosis for measles and rubella and epidemiology of measles in the Philippines. In 2010, the highest number of samples (n = 2626) was referred in March and the highest number of positive samples was also detected in March (n = 785). Among 16 regions in the Philippines, the National Capital Region (NCR) showed the highest number of measles-positive cases in 2010. Children under three years had the highest incidence of measles in 2010. The incidence of measles saw a resurgence from 2007 after very low incidence during 2004–06.

For internal quality control, the laboratory implemented in-house control samples for both measles and rubella IgM ELISA tests. Virus isolation was conducted for 119 samples, RT-PCR for 19 samples and immunofluorescence for 12 samples respectively in 2010. Confirmatory samples were sent to the Hong Kong RRL and results of confirmatory testing in 2010 showed good concordance. Genotyping of measles and rubella was performed at Hong Kong RRL and the genotypes detected were D9 and G3 (Region 6) for measles and 1j for rubella in 2010.

2.2.7 Republic of Korea

Ms Hee Sook Yoon from the Korea Center for Disease Control and Prevention (Korea CDC) introduced the laboratory testing methods and national laboratory data for 2008–2010. This laboratory performs not only IgM but also IgG ELISA for measles and rubella, viral isolation and RT-PCR. It also performs differential diagnosis of parvovirus B19 and HHV6 using IgM ELISA. Proficiency testing, confirmatory testing and the algorithm for testing suspected measles/rubella cases were also reviewed together with recent laboratory data. In 2008 and 2009, 22 out of 165 reported cases and 32 out of 132 reported cases tested positive for measles, while 112 out of 279 reported cases tested were positive for measles in 2010.

In 2010, a measles outbreak was reported in one middle school located in Incheon metropolitan city. Among samples from 112 measles-positive cases, 96 were IgM-positive, 40 were RT-PCR-positive and virus was isolated from 18 samples. The genotyping result showed H1 strains in all cases from this outbreak.

From 2008 to 2010, 23%–46% of samples were positive for HHV6 IgM and 9%–19% for parvovirus IgM. For the quality assurance of laboratories involved in measles IgM testing, this laboratory also prepared and distributed Measles/Rubella Proficiency Panel samples for 12 provincial public health laboratories and four private diagnostic laboratories.

2.2.8 Singapore

Dr Lui Sook Yin from Singapore General Hospital’s Department of Pathology presented virological testing methods used in the laboratory. He also presented quality assurance measures including laboratory accreditation, use of in-house control and equipment monitoring and maintenance, plus the epidemiology of measles and rubella in Singapore. The number of reported measles cases has rapidly declined since the introduction of compulsory measles vaccination in August 1985. The incidence of measles has remained at a low level since 1998 since the catch-up immunization in 1997 and introduction of the two-dose MMR vaccination strategy in 1998. A national serosurvey in 2005 showed an overall seroprevalence of 96.7% for measles and 87.4% for rubella among adults of 18–74 years. It was noted that 15.8% of females of 18–44 years of age remained susceptible to rubella infection. There was no reported congenital rubella case and termination of pregnancy in 2009 due to rubella infection. During January–October 2010, this laboratory tested 159 samples for measles IgM and 701 samples for
rubella IgM. The WHO algorithm for measles and rubella testing is not followed due to a shortage of funding to support additional rubella or measles testing. From 2007 to October 2010, 24 measles strains were sequenced. D5 (2007 and 2008), D9 (2007, 2008, 2009, 2010), D4 (2008), H1 (2009 and 2010), D8 (2009) and G3 (2010) were detected. Two G3 cases in 2010 were related to travel to Indonesia. The remaining challenges for this laboratory were: (1) tests were performed based on clinicians’ requests, and (2) clinical and epidemiological information was not available for most cases.

2.2.9 Viet Nam

Ms Trieu Thi Thanh Van from the National Institute of Hygiene and Epidemiology (NIHE) presented the national measles immunization programme and laboratory testing of measles and rubella in northern Viet Nam. The country introduced two doses of measles vaccination for children of 9–11 months and six years in 2006. From 2010, the schedule of the second dose of measles vaccine changed to 18 months. From September–November 2010, a national measles vaccination campaign was conducted for children under six years old.

This laboratory receives measles and rubella kits from both WHO (Siemens) and the Ministry of Health (Biorad). In 2009, 2187 (56%) out of 3890 samples tested were positive for measles IgM. In 2010, 225 (29%) out of 788 samples tested were measles IgM-positive. Out of 4050 samples tested for measles in 2009, 2315 were tested within seven days, and out of 1067 samples tested in 2010 (January–November), 930 (87%) were tested within seven days. Out of 2208 and 867 samples tested for rubella in 2009 and 2010, 545 (25%) and 389 (45%) respectively were positive for rubella. This laboratory performs virus isolation using throat swab samples. In 2009 and 2010, 23 out of 106 and one out of nine virus isolation samples were CPE-positive and confirmed by measles RT-PCR.

Ms Dang Thanh Giang from the Pasteur Institute in Ho Chi Min City presented measles and rubella testing in southern Viet Nam. This laboratory performs viral isolation, IgM ELISA and RT-PCR sequencing for genotyping. Serum and throat samples are collected 4–28 days and 0–7 days after onset respectively. In 2009 and 2010, 2181 samples tested were positive for measles IgM and 877 samples tested were positive for measles IgM. Among samples tested for rubella IgM, 985 were positive in 2009 and 1523 were positive in 2010. Among measles-positive cases, 51% were 0–6 years old and among rubella-positive cases, 60% were 7–24 years old.

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. Two groups shared one ABI 9700 Thermocycler for RT-PCR:

(1) Group A – China (2)
(2) Group B – the Philippines (2)
(3) Group C – Viet Nam (2)
(4) Group D – Malaysia (1) and Singapore (1)
(5) Group E – the Republic of Korea (1) and New Zealand (1)
The first day of practical sessions on 22 November 2010 included Session (1) RNA extraction of measles and rubella from infected cells. Each group was given five samples. For measles, two urine samples and one culture fluid sample, and for rubella, one urine sample and one culture fluid respectively were used. Qiagen QIAamp Viral RNA mini kits were used for extraction of RNA.

Session (2): Rubella detection and genotyping RT-PCR was performed using templates provided by US CDC. Separate rooms were used for master mix preparation, adding RNA templates and handling PCR products. Qiagen One-Step RT-PCR kit and RNase inhibitor (ABI) were provided.

The second day of practical sessions was in RT-PCR for measles genotyping and gel electrophoresis of rubella detection and genotyping RT-PCR, followed by PCR purification for measles and rubella. Qiagen One-Step RT-PCR kit was used for measles genotyping RT-PCR. Gel electrophoresis of RT-PCR products of rubella was conducted using 2% gels prepared and provided to participants. Gel electrophoresis for measles RT-PCR was also conducted. Qiagen QIAquick PCR Purification kit was used to purify the PCR products. Electrophoresis of post-purification products of measles and rubella was done to determine the amount of DNA templates to be added for cycle sequencing the next day.

On the third day, master mix for cycle sequencing was prepared using Big Dye Terminator v3.1. Purification of cycle sequencing products was performed, followed by sequencing runs for measles and rubella.

The fourth day of practical sessions focused on sequence analysis for genotyping of measles viruses. Dr Paul Rota gave a presentation on "Introduction to sequence analysis and quality control of molecular tests". He emphasized quality control for molecular tests including template quality and quantity, primer quality, reviewing of chromatogram and sequence data validation. Detailed explanations and tips were given to participants on reviewing sequence data, sequence quality control and data management. This included importing data from chromatograms, preparing alignment, determining genotypes and performing basic local alignment search tool (BLAST) search on GenBank. For laboratories without sequencing facilities, options to submit PCR products for sequencing were presented. PCR products (either purified or unpurified) can be shipped without drying and are stable for at least one month at room temperature. Precautions for working with RNA and avoiding template contamination were presented. Full explanation was given on molecular proficiency testing (PT) for measles. Genotype and sequence data reporting to WHO HQ and MeaNS was explained, followed by a practical session on using MeaNS.

On the last day, a data management and reporting session discussed monthly reporting of laboratory data to WHO’s Western Pacific Regional Office using the newly revised Microsoft Access format. Genotype and sequence data submission to WHO HQ and MeaNS were also covered.

Dr Joseph P. Icenogle gave a presentation on sequence analysis for rubella. This included detailed hands-on practice in sequence analysis for rubella virus genotyping, reviewing sequence data and sequence quality control. It also included importing data from chromatograms, preparation of alignment, determination of genotypes and performing BLAST search on GenBank.
3. CONCLUSIONS

3.1 General

The two main objectives of the training were fully achieved during 5.5 days of intensive hands-on practical sessions, lectures and group work. By the end of the workshop, the technical capacity and knowledge of all participants had been enhanced. They were able to understand and perform molecular detection of measles and rubella viruses by RT-PCR and sequencing and laboratory quality assurance of measles and rubella diagnosis. All participants were further familiarized with data management using the WHO measles and rubella laboratory data reporting format for reporting to the Western Pacific Regional Office.

3.2 Workshop evaluation

The participants were positive in their feedback; the workshop met its objectives. Administrative arrangements for the workshop by PHLC were excellent. A locker was allocated to everyone during the workshop and instructions to go to the laboratory or lecture room for each session were clearly given to the participants by PHLC staff.

Participants were encouraged to contact each other, the facilitators and WHO’s Western Pacific Regional Office to follow up on practical issues such as quality assurance. This would include reporting of molecular PT results, in-house control samples, confirmatory testing, genotype data management and reporting, and how to strengthen the molecular detection capacities in each network laboratory.

With full support from PHLC staff, the training ran smoothly throughout the practical and lecture sessions. Several PHLC staff provided full support for each group. Allocating different rooms for different procedures and moving to different rooms was well organized. The participants efficiently completed all the practical sessions and understood issues addressed during the training. Between practical sessions, calibrating micropipettes using the specific calibration programme at PHLC was demonstrated. Participants also had a chance to participate actively in calibration of micropipettes.

The training schedule provided adequate time for performing practical procedures at a reasonable pace and for information sharing among the participants. The duration of each presentation also allowed adequate 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 and laboratory quality assurance of measles and rubella diagnosis. By the end of the workshop, participants understood the procedures of molecular detection and data management and reporting using the new laboratory reporting format (Microsoft Access).

Participants were familiarized with the requirements for reporting genotype and sequence data to WHO HQ and MeaNS.

At the end of the workshop, the WHO Measles and Rubella IgM Proficiency Testing Panel samples and molecular proficiency samples for measles and rubella were distributed to participants.
3.4 Follow-up to the workshop

Participants were requested to report the results of IgM Proficiency Panel (PT) samples within 14 days of the samples arriving in the laboratory. Results of the molecular practice PT samples were requested to be submitted by 1 February 2011. Most laboratories that participated in the training reported results within the agreed time-frame.

The results of the Measles Rubella IgM Proficiency Panel samples were received from participating network laboratories. Most laboratories used Siemens kits, and some used other kits such as Virion Serion, Haitai (China) or Denka Seiken (Japan). Results were finalized by VIDRL and feedback was immediately sent to all laboratories. All laboratories passed the WHO PT and all obtained 100% for measles, but seven laboratories obtained 95% and two 90%.

The results of molecular PT samples were supposed to be received by 1 February, but some laboratories did not submit results within the agreed time-frame. As the Philippines and Malaysia did not have a DNA sequencer, the PCR products were sent to PHLC Hong Kong. Sequencing of those products was conducted at PHLC and sequence data were sent back to the two laboratories for editing and sequence analysis. After the data received from Hong Kong were further analysed by the two laboratories, they submitted dendrograms to US CDC. US CDC provided a summary of molecular PT results based on the results received from participating laboratories.