Seventeenth Meeting of the Technical Advisory Group on Immunization and Vaccine Preventable Diseases in the Western Pacific Region

LABORATORY WORKSHOP

Manila, Philippines
7-9 July 2008
REPORT

SEVENTEENTH MEETING OF THE TECHNICAL ADVISORY GROUP ON IMMUNIZATION AND VACCINE PREVENTABLE DISEASES IN THE WESTERN PACIFIC REGION

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NOTE

The views expressed in this report are those of the participants of the Laboratory Network Workshop of the seventeenth meeting of the Technical Advisory Group on Immunization and Vaccine Preventable Diseases in the Western Pacific Region and do not necessarily reflect the policies of the World Health Organization.

This report has been printed by the Regional Office for the Western Pacific of the World Health Organization for the participants in the Laboratory Network Workshop of the seventeenth meeting of the Technical Advisory Group on Immunization and Vaccine Preventable Diseases in the Western Pacific Region, which was held in Manila, Philippines, 7-9 July 2008.
SUMMARY

Representatives of the WHO-designated regional poliomyelitis and measles/rubella laboratories and of the Japanese encephalitis laboratory from selected countries met in Manila from 7 to 9 July 2008. The meeting also was attended by the Technical Advisory Group members, temporary advisers from the United States Centers for Disease Control and Prevention (CDC), representatives from PATH, WHO Headquarters laboratory coordinators, and WHO Western Pacific Regional Office and EPI country staff.

The objectives of the meeting were:

(1) to review the laboratory performances of Regional Reference Laboratories (RRLs) and national laboratories for polio and measles (NPLs), to maintain polio-free status and to reach the goal of measles elimination in the Region;

(2) to discuss the introduction of a new algorithm for poliovirus identification in the Region and new requirements for the measles laboratory network in the Region; and

(3) to establish the Japanese encephalitis LabNet for selected countries and to develop laboratory diagnostic capacities for JE in the Region.

The Global Polio Laboratory Network (GPLN) comprised of national, regional reference, and global specialized laboratories plays a very crucial role in the global polio eradication initiative. Besides timely identification of wild polioviruses, the rapid detection of vaccine-derived poliovirus (VDPV) that causes acute flaccid paralysis is becoming increasingly important because of polio outbreaks reported due to circulating VDPV. Since the Regional Polio Laboratory Network meeting in 2002, there have been new developments in the GPLN. A new standard WHO algorithm for poliovirus isolation and identification has been introduced into the network and should provide the results of primary isolation within 14 days of the receipt of sample in the laboratory. Further, the results of identifying a wild type or vaccine strain of poliovirus with the use of an intratypic differentiation (ITD) testing procedure should be available within seven days. Participants were briefed about these changes. Representatives from the polio network laboratories presented the country data for the period 2002-2008.

Recommendations for the polio laboratory network include: (1) the new checklists for the NPLs and RRLs should be implemented among network laboratories, including 31 provincial laboratories in China, as soon as possible. Standard operating procedures (SOPs) also should be revised to meet new requirements in the checklists; and (2) NPLs are encouraged to implement the new algorithm for virus isolation but can continue to perform the polio neutralization test in parallel to shipping the isolates to the RRL. SOPs and databases in each laboratory should be revised and implementation of the changes should be reported to the regional laboratory coordinator.

Since the measles and rubella laboratory network was established in 2004, the LabNet has played a critical role in the progress towards achieving the regional goal of measles elimination by 2012. The LabNet tested more than 110,000 serum samples in 2007 in the Western Pacific Region. All Western Pacific Region NPLs passed the proficiency test in 2007, and confirmatory testing has been implemented by many national laboratories. To cope with the increased number of confirmatory samples in the region, the national measles
A laboratory in Hong Kong (China) was designated as a regional reference laboratory in May 2007. Participants discussed remaining challenges for the WPR LabNet, including improving sample collection for virus detection, validation of test kits used in the subnational LabNet, and the improved timeliness and completeness of laboratory reporting. Also discussed were new requirements for the measles LabNet, including the introduction of new case-based laboratory reporting and revised accreditation checklists emphasizing the timeliness of reporting. The participants also presented their national data for the period 2004-2008.

Recommendations for the measles/rubella laboratory network include: (1) a new case-based laboratory reporting scheme should be used in all measles/rubella network laboratories (NMLs) as much as possible and network laboratories were asked to submit their monthly data for the previous month by the 10th of the month; (2) as recommended by the Global Measles Laboratory Network Meeting of September 2007, new checklists for the NMLs and RRLs that emphasize the timeliness and completeness of genotyping and sequencing of measles viruses should be used for the accreditation of network laboratories. NMLs were encouraged to submit genotype information about circulating measles and rubella strains to the WHO genotype database. This genotypic information should be shared with the regional laboratory coordinator; (3) a regular confirmatory testing mechanism for measles and rubella samples should be established for all NMLs in the Region to ensure the accuracy and quality of testing. The confirmatory results should be shared with regional laboratory coordinators. The reasons for discrepancies between the NMLs and the RRLs in the results should be sought and immediate corrective actions should be taken in the NMLs; and (4) a mechanism for sample referral among the Pacific island countries should be reviewed and reestablished in the Region. Some mechanisms to support the NMLs in priority countries should be established and maintained in the Region.

The Western Pacific Region has seven countries either known to be endemic for Japanese encephalitis (JE) or suspected to be endemic for JE. These countries include China, Cambodia, the Lao People's Democratic Republic, Malaysia, Papua New Guinea, the Philippines, and Viet Nam. However, the activities of laboratories are limited among these JE-endemic or suspected JE-endemic countries in the Region. Therefore, it was proposed to create a laboratory network for JE to improve the capability of JE case confirmation in the Region.

Recommendations for the JE laboratories in the Region include: (1) potential GSL (one), RRLs (two), and national laboratories for JE should be identified by 2008. A formal accreditation system to evaluate the laboratory performances of the network laboratories should be established in collaboration with WHO HQ and the WHO South-East Asia Regional Office (SEARO); (2) proficiency test panels for JE should be arranged for the network laboratories by 2009; (3) a training workshop for the laboratory diagnosis of JE should be organized by early 2009; and (4) laboratory capability should be established to support acute encephalitis syndrome surveillance to detect bacterial antigens as well as JE.

It was recommended that two additional laboratory network coordinators are needed for the Region, one with expertise in virology and another with expertise in bacteriology. They would be necessary to deal with the increasing number of specimens, the heightened activities of the polio and measles laboratory networks and the introduction of laboratory based surveillance for new vaccines in the Region.
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1. INTRODUCTION

1.1 Background information

1.1.1 Poliomyelitis Laboratory Network

The regional poliomyelitis laboratory network of 43 laboratories is well established and is being used to provide essential information about responding to the importation of wild poliovirus and detection of vaccine-derived polioviruses (VDPV). Performance levels have been maintained according to the requirements for certification of poliomyelitis eradication since the Region was declared poliomyelitis-free in October 2000.

The performance of network laboratories is monitored through a laboratory accreditation programme that is administered by the World Health Organization (WHO). Elements of the programme are proficiency testing, onsite performance reviews, monitoring of accuracy, and timeliness of reporting. The formal system for annual accreditation of network laboratories is well established. All laboratories in the Regional Laboratory Network are performing at WHO accreditation standards.

However, concern has been expressed about the challenges of maintaining certification standards for reporting and investigating acute flaccid paralysis (AFP) cases and collecting adequate stool specimens. Priorities may have been directed to other public health activities after certification and complacency evolved following the interruption of wild poliovirus transmission in the Region.

Rapid confirmation of the transmission of wild polioviruses and VDPVs is an essential first step towards implementing interventions to prevent virus spread. The Global Polio Laboratory Network (GPLN) has formulated a strategic plan to reduce laboratory reporting times without compromising poliovirus detection sensitivity.

Key elements of the plan include introducing a new test algorithm that has been proven through field evaluation in three locations and subsequent implementation in three polio endemic WHO regions (Africa, Eastern Mediterranean, and South-East Asia). It reduced reporting times by 50% without compromising poliovirus detection sensitivity and increased the number of facilities with onsite capacity for virus isolation and intratypic differentiation (ITD). The new plan also reduced the need for the intercountry shipment of virus isolates for analysis and worked out new diagnostic procedures and reagents.

The main operational challenges associated with using the new test algorithm are the increased workload and costs of ITD tests and the availability of sufficient enzyme-linked immunosorbent assay (ELISA) reagents to meet demands. Adjustments have been made to the GPLN’s accreditation and quality assurance programme, including the revision of the WHO Polio Laboratory Manual. The GPLN were expected to meet the new targets for reporting times starting in January 2008. New proficiency test panels were to be distributed in 2008 to laboratories implementing the new algorithm.

The GPLN were looking into poliovirus surveillance needs. Enterovirus surveillance was discussed as one non-AFP-based approach in polio-free areas to assess its relevance and comparative sensitivity to using AFP.
To maintain excellent performance levels, it is crucial to standardize the laboratory data management system, to establish a support system for equipment maintenance and the distribution of selected supplies, and to draw up a standard approach to in-house quality control. Additional support and assessment also are required to meet the continuing demand for training in basic laboratory techniques.

1.1.2 Measles and Rubella Laboratory Network

The WHO Western Pacific Region established a measles elimination target date of 2012. The first laboratory meeting for the measles/rubella laboratory network was held in 2004. The strength of the measles and rubella laboratory network (LabNet) is the establishment of standardized testing and reporting procedures. Most laboratories use well-validated IgM assays and follow standardized validation procedures, with the staff having participated in WHO-coordinated training.

An annual proficiency testing programme has been in place in the Western Pacific Region since 2001. During the period 2006-2007, laboratories in all six WHO regions participated in the same proficiency testing programme for the first time. A comprehensive revision of the 1999 Measles Laboratory Manual was published in August 2008. It increased the emphasis on rubella diagnosis, cell culture, virus isolation, quality assurance, and progress towards meeting programme goals.

The resource needs to maintain the LabNet are increasing with the expanding number of laboratories and workload. A very limited number of partners support the LabNet, and there is major dependence on the United States Centers for Disease Control and Prevention (CDC) for financial support. A few Global Specialized and Regional Reference laboratories give technical support and coordinators in each of the regions and at WHO Headquarters harmonize activities.

The Western Pacific Region LabNet is established and more than 110,000 serum samples were tested in 2007, about 100,000 of which were in China. Measles outbreaks in Japan and the Republic of Korea in 2007 were confirmed by the designated measles laboratory at the National Institute for Infectious Diseases (NIID), Tokyo, and the Korea Centers for Disease Control and Prevention, Seoul (KCDC). Genotypes were confirmed as D5 in Japan and H1 in the Republic of Korea. Laboratory confirmation of measles cases in China and Japan was strengthened during 2008. However, the national measles laboratories in the Lao People's Democratic Republic and Pacific island countries are not yet fully functional.

All Western Pacific Region national laboratories passed the proficiency test in 2007 and confirmatory testing has been implemented by many national laboratories. Training workshops were held at the Victorian Infectious Diseases Reference Laboratory, Melbourne (VIDRL), in May 2005 and in the Hong Kong (China) laboratory in March 2006 for ELISA and virus culture techniques. They also were conducted at the Chinese Center for Disease Control and Prevention, Beijing (China CDC), for China's 31 provincial laboratories during the period 2005-2007. Remaining challenges for the Western Pacific Region LabNet include improving sample collection for IgM and virus detection, validation of test kits used in subnational LabNets, and timeliness and completeness of laboratory reporting. Discussions also focused on new requirements for the measles laboratory network, including the introduction of a case-based laboratory reporting scheme and revised accreditation checklists emphasizing the timeliness of reports.
1.1.3 Japanese Encephalitis Laboratory Network (JE LabNet)

The Western Pacific Region has seven countries either known or suspected to be endemic for Japanese Encephalitis (JE). They include China, Cambodia, the Lao People's Democratic Republic, Malaysia, Papua New Guinea, the Philippines, and Viet Nam. In Japan and the Republic of Korea, the disease largely has been controlled through universal vaccination programmes for least four decades.

However, the activities of laboratories are limited among the known or suspected JE endemic countries in the region and JE is not perceived to be a priority public health problem in the Lao People's Democratic Republic, the Philippines, and Papua New Guinea. The latter slowed the implementation of these activities with the concurrence of the Ministry of Health. The South-East Asia Regional Office (SEARO) has established a JE LabNet and worked out some capacity problems in most countries in the Region. However, the Western Pacific Regional Office has not established a JE LabNet. There is an urgent need to create one and to improve laboratory capacities in the Region in order to get a better estimate of the JE disease burden and the impact of disease control measures.

1.2 Objectives

The objectives of the meeting were:

(1) to review the laboratory performances of Regional Reference Laboratories and national laboratories for polio and measles, to maintain polio-free status, and to reach the goal of measles elimination in the Region;

(2) to discuss the introduction of a new algorithm for poliovirus identification and new requirements for the measles laboratory network, both in the Region; and

(3) to establish the JE LabNet for selected countries and to work out laboratory diagnostic capacity for JE in the Region.

1.3 Appointment of workshop officers

On Day 1, Dr Olen Kew of CDC was appointed as chairperson, Dr Hiroyuki Shimizu of NIID, Tokyo, as Vice-Chairperson and Dr Bruce Thorley of VIDRL, Melbourne, as Rapporteur.

On Day 2, Dr Tashiro Masato of the NIID was appointed as chairperson, Dr Paul Rota of CDC as Vice-Chairperson, and Dr Wilina Lim of the Public Health Laboratory Center, Hong Kong (China), as Rapporteur.

On Day 3, Mr David Featherstone and Dr Esther de Gourville of WHO Headquarters were appointed as chairpersons, Dr Zainah Saat of the Institute of Medical Research, Malaysia, as Vice-Chairperson and Dr Asheena Khalakdina of the Programme for Appropriate Technology in Health (PATH), Seattle, as Rapporteur.
2. PROCEEDINGS

2.1 Progress and Challenges of Global/Regional Laboratory Network

2.1.1 Status of the global polio laboratory network

Dr Esther de Gourville, Global Polio Laboratory Network Project Leader, coordinator of the Global Polio Laboratory Network (GPLN), gave an overview of the GPLN.

Since the World Health Assembly resolution to eradicate polio in 1988, polio-endemic countries have been reduced from 125 to four countries and the transmission of wild poliovirus type 2 has been interrupted since 1999. In 2007, 1313 cases were reported in 12 countries and 92% were from four endemic countries. Only types 1 and 3 have been detected in ratio of 1:3 among reported cases. Tracing virus transmission pathways using VP1 nucleotide sequence data during the period 2003-2008 showed that viruses of Indian origin travelled to Africa. Viruses of Nigerian origin also travelled to other countries in Africa and to countries in two other WHO regions (Eastern Mediterranean and South-East Asia). The GPLN has screened viral isolates to detect programmatically important Vaccine Derived Polio Viruses (VDPVs) since 1999. By 2007, 32 313 cases were screened and 176 circulating vaccine-derived polioviruses (cVDPVs), 15 immunodeficient vaccine-derived poliovirus (iVDPVs), and 20 ambiguous VDPVs (aVDPVs) were detected. Type 2 cVDPVs were reported from Nigeria from 2007, with 94 cases detected up to June 2008.

The new test algorithm for poliovirus detection and intratypic differentiation has been implemented successfully in three polio-endemic regions, resulting in a 50% reduction in time to confirm wild poliovirus in laboratories. New accreditation checklists were introduced in the GPLN beginning in January 2008. Real time polymerase chain reaction (PCR) assays for ITD and VDPV screening were being evaluated in three laboratories. The development of audiovisual training materials for a biorisk campaign in LabNet was ongoing.

Areas requiring more attention would be: (1) communication to improve timeliness or reporting and follow-up of cell sensitivity testing; (2) expansion of ITD capability; and (3) establishment of accreditation criteria for labs performing sequencing, including timelines and guidelines on dealing with increasing genetic diversity.

2.1.2 Global Measles Rubella Laboratory Network

Mr David Featherstone presented updates of the global measles/rubella laboratory network. Progress has been made with the global genotype database, and data taken during the period 1979-2008 were put into an Excel format. All countries were encouraged to submit their genotype data monthly. However, only four countries in the Western Pacific Region have put their measles sequences in this database and two countries submitted their rubella sequences. Also discussed were major measles virus transmission pathways, including the predominance of D5 strains that caused widespread outbreaks globally in the past three years.

Alternative sampling methods for measles and rubella, such as dried blood spot (DBS) or oral fluid, were discussed. These methods have now been validated comprehensively by the LabNet. They can be used to improve surveillance in logistically challenging countries and territories and to provide enhanced molecular surveillance capabilities.
Because of the challenges and resource problems that include an increasing workload for confirmatory testing and limited partners supporting LabNet, it is strongly recommended that the Expanded Programme on Immunization (EPI) incorporate lab costs in the surveillance programme. Other challenges facing LabNet include improving the timeliness of molecular surveillance reporting and the completeness of molecular surveillance, obtaining epidemiological data in combination with molecular data, and integrating the laboratory database with cases identified through the surveillance programme.

2.1.3 Progress and challenges of polio and the measles laboratory network in the Western Pacific Region

Major developments since the last Regional Polio Laboratory Network meeting in 2001 were presented by Dr Youngmee Jee, regional EPI Laboratory coordinator. The network tested stool specimens from about 39 064 AFP cases during the period 2002-2007. In 2006, the national polio laboratory in Singapore detected a type 1 wild poliovirus from a Nigerian child who visited Singapore for medical treatment. In July 2007, the reference laboratory in Australia isolated South Asia (SOAS) WPV1 from an adult patient of Pakistani origin who experienced limb weakness while visiting Pakistan before entering Australia. There was no evidence of a local spread of this virus to household or travel contacts. With extensive VDPV surveillance in China, type 2 cVDPVs were detected in 2004 and 14 aVDPV strains were reported during the period 2006-2007. In Cambodia, type 3 cVDPVs were detected during the period 2005-2006.

In the Western Pacific Region, all regional reference laboratories (RRLs), national polio laboratories (NPLs), and provincial laboratories in China, except Tibet, are fully accredited and operating under WHO standards. The NPL in Papua New Guinea has not been functional since August 2007 but has agreed to send AFP stool samples to the RRL in VIDRL, Melbourne, for testing.

The timeliness of reporting laboratory results has improved in the Region. Average days from the receipt of samples to primary culture results were 21.4 in 2006, 21.8 in 2007, and 19 in 2008 (as of week 24). Average days for ITD were 34.5 in 2006, 15.6 in 2007, and 13.1 in 2008.

All network laboratories, including the 31 provincial laboratories in China, passed the proficiency test for poliovirus isolation in 2007 and 2008 with a 100% score. Cell sensitivity testing of RD cells and L20B cells for polioviruses was implemented regularly in the network.

There is an increasing use of analysis of VP1 nucleotide sequences for ITD and poliovirus characterization in the Region because of a shortage of ELISA reagents for ITD. There is an urgent need to establish a standardized protocol and proficiency testing for PCR-sequencing ITD. There will be a need for laboratory training workshops in the Region in anticipation of the introduction of real time PCRs for ITD and VDPV screening. Sharing the cell sensitivity result in a timely manner is crucial, and it is recommended that network laboratories report cell sensitivity results within 48 hours of test completion. This recommendation also applies to the provincial laboratories in China and should be emphasized during the annual polio laboratory training in China.

In accordance with the implementation of the timeline of reporting ITD results within seven days, all ITD laboratories were required to comply with this requirement in 2008. A regional ITD training workshop was planned for 2009 with additional NPLs being designated to perform ITD functions.
The Western Pacific Region measles/rubella LabNet performs critical functions for measles elimination by confirming or discarding suspected measles cases, identifying measles genotypes, and determining potential geographic routes of transmission. The LabNet was proposed in the 2001 Technical Advisory Group (TAG) meeting and has grown to 382 laboratories, including one global specialized laboratory (Japan), three RRLs (Australia, China, Hong Kong (China)), 16 national laboratories and, in China, 31 provincial and 331 prefecture laboratories. Quality assurance for the laboratories is conducted through regular onsite visits to evaluate performance, the parallel testing of a sample of positive and negative specimens at RRLs, and annual proficiency tests. As of June 2008, two of four global specialized labs or RRLs and nine of 16 national laboratories were accredited in the Region. An assessment of the remaining laboratories was to be completed in the next year. All regional and national network laboratories and 29 to 31 provincial laboratories in China passed the 2007 measles proficiency test.

As of July 2008, most countries in the Region were reporting case-based measles surveillance data to the Regional Office for the Western Pacific. The Regional Office is working closely with countries to encourage regular and timely reporting. Case-based measles surveillance in elimination settings requires laboratory support for confirmation of the clinical diagnosis by identifying measles-specific IgM-antibodies and/or the identification of the measles virus in appropriate clinical specimens.

To improve data reporting from the laboratory network, a new case-based reporting format for laboratories has been established and has been in use since January 2008. As of July 2008, ten of 20 laboratories are reporting data monthly using the new format. There is an urgent need to improve communications with measles laboratories in Pacific Island Countries and areas. The LabNet evaluates a large number of specimens annually. In 2007, more than 110,000 serologic specimens were tested for measles IgM, about 100,000 of which were in China.

Despite improved surveillance for measles and rubella strains, more information of circulating genotypes should be collected from countries. Recently, genotype H1 strains have been most commonly detected in the Region, including China, Hong Kong (China), and the Republic of Korea. Genotypes of measles virus identified in the Region since 2005 include A1, A2, D4, D5, D8, D9, G3, and H1.

2.2 New Developments of Polio Laboratory Network

2.2.1 Description and justification for use of a new test algorithm for more rapid poliovirus confirmation

Dr Esther de Gourville described the rationale for using a new test algorithm for more rapid poliovirus confirmation and its implications and some experiences with it. The main differences between the traditional and new test algorithms are: (1) shortened cell culture step; (2) changes in how cytopathic effect (CPE) positive cultures are passed; (3) omission of routine serotyping before ITD; and (4) passage of all L20B isolates into RD cells before ITD. Poliovirus serotype and intratype are simultaneously determined by PCR followed by immediate reporting of poliovirus with a non-Sabin-like (NSL) reaction and referral for sequencing. Monotypic Sabin-like viruses subsequently are tested by ELISA. There is a separation of poliovirus mixtures by neutralization tests before performing ELISA on separated viruses.

Implications of new test algorithms for laboratories were described. National laboratories will need to update standard operating procedures (SOPs), and change
worksheets and database and cell culture preparation cycles. There will need to be changes in the materials referred to ITD laboratories. National laboratories do not need to perform serotyping before they refer the samples to ITD laboratories. Functioning ITD laboratories also need to update SOPs, worksheet, and database and report the results within seven days. The new test algorithm has been implemented successfully in polio-endemic regions with a 50% reduction of reporting time. Laboratories in non-endemic regions or countries may implement the new algorithm where there is interest, existing ITD capability, and sufficient resources.

2.2.2 Mechanisms used and status of implementation of the new test algorithm in polio-endemic regions

Dr Fem Paladin discussed the mechanisms used and the status of implementation of the new test algorithm in the WHO African Region, Eastern Mediterranean Region, and South-East Asia Region. Key activities included the orientation of laboratory directors to the new test algorithm at regional laboratory meetings between July and December 2006, ITD training workshops, and the improvement of the infrastructure of additional laboratories to upgrade their ability to perform ITD testing. Also included was the revision of SOPs and test worksheets, the orientation of surveillance staff and data management personnel, and the revision of databases and the reporting procedures in laboratories and regional offices.

Following the Global Polio Meeting in 2007, a revised supplement to the Polio Laboratory Manual, which describes the new test algorithm, was distributed in October 2007. A follow-up training workshop in cell culture techniques was conducted in the African Region in April 2008 to enhance the implementation of the new test algorithm. By December 2007, all 44 laboratories in polio-endemic regions had implemented the virus isolation algorithm and 26 of these laboratories had the capability for both virus isolation and ITD testing.

Among non-endemic regions, only laboratories in the Region of the Americas have implemented the new test algorithm. Laboratories in the European Region were briefed on the new test algorithm in November 2006. This meeting provided an opportunity for laboratories in the WPR to be oriented to the new algorithm.

2.2.3 New real time PCR assays for ITD and VDPV screening

Dr Olen Kew from CDC presented updates on new real time PCR assays for ITD and VDPV screening, which were formulated by the CDC. The standard CDC diagnostic PCR has been adapted to a real time ITD PCR. Advantages of a real time PCR for ITD would be increased sensitivity and specificity, applicability to virus mixtures, elimination of product analysis by gel electrophoresis, decreased risk of cross contamination, and providing all data in electronic form. Disadvantages would be the use of degenerate primers and probes that could be more sensitive to minor variations in conditions, the incompatibility of some real time instruments with the reporter dyes used, and high equipment costs.

In addition, real time PCR for VDPV screening has been developed to screen possible VDPVs by targeting VP1. This assay could replace ELISA for screening of Sabin-like viruses following PCR ITD. The evaluation has been completed with more than 1000 poliovirus isolates. Parallel testing with ELISA has shown a higher sensitivity to cVDPV detection.
2.2.4 Experience with the new test algorithm and molecular detection methods at VIDRL

Dr Bruce Thorley presented his experience with the new test algorithm and molecular detection methods used in the VIDRL. Since the new algorithm was presented at the 12th GPLN meeting in 2006, the VIDRL reduced the cell culture observation from 14 days to 10 days beginning in July 2006. Observation was maintained for 10 days before reporting as negative for virus isolation. Passage of L20B + to RD was implemented beginning in October 2007. The VIDRL has used PCR and VP1 sequencing from 2001 as ITD methods and replaced ELISA since 2005. Dr Thorley also presented the analysis data from a wild poliovirus importation case in 2007. Testing by cell culture, ITD (PCR, ELISA), VP1 sequence was completed within seven days of the receipt of samples. The phylogenetic analysis of VP1 sequence provided an epidemiological link to the source of infection.

2.2.5 New developments and requirements of polio laboratory network in the Western Pacific Region

Dr Youngmee Jee presented major events and developments, including importation of wild poliovirus to Singapore in 2006 and to Australia in 2007. He also discussed changes in new accreditation checklists and the polio laboratory manual with two supplements that included a new algorithm and adaptation of newly received cells to local conditions. Both old and new algorithms are listed in the new checklists and cell sensitivity testing replaces internal quality control procedures. The results should be reported within 48 hours of completion.

During the period January 2006-May 2008, the mean time for virus isolation was reduced from 70.09 days to 31.70 days and the mean time for ITD was cut from 49.19 days to 13.07 days. The regional workload of ITD laboratories with both virus isolation and ITD (six of 43 laboratories) remained at a low level (4%–7%) since virus isolation work is conducted mostly in provincial laboratories in China.

Cell sensitivity testing results of all network laboratories, including provincial laboratories in China, were reviewed for the period June 2007-May 2008. Problems of low titre or fluctuation of titres showed up in some laboratories. The importance of reporting the cell sensitivity result in a timely manner (within 48 hours of completion) was emphasized. The WPRO will use the traditional algorithm for 2008 proficiency testing for poliovirus isolation.

During the meeting, ITD PCR panels were distributed to Australia, Hong Kong (China), New Zealand, and Singapore.

2.2.6 Country reports

2.2.6.1 Australia

VIDRL is the national polio laboratory for Australia, Brunei Darussalam, the Pacific Island Countries and Papua New Guinea (from August 2007) and serves as a regional reference laboratory for the Western Pacific Region.

The polio reference laboratory was established in 1994 and coordinates AFP surveillance. During the period 2002-2008, 71 Sabin-like strains, 110 nonpolio enterovirus (NPEVs) and 419 NEV strains were detected. From the period 1996-2007, stool samples from 193 AFP cases were tested and enteroviruses were isolated from 31 cases: wild poliovirus type 1 (importation in 2007), Sabin type 1, 2, 3, Echovirus types 9, 11, 18, 30,
Coxsackievirus A4, A24, and B5, and Enterovirus 71 and 75. NPEV isolation rates from stool specimens from AFP cases were about 5% during this period.

From the Pacific island countries and areas, specimens were referred from Fiji, Kiribati, the Marshall Islands, New Caledonia, Solomon Islands, and Tonga. The NPEV isolation rate was 20.6% among 204 stool samples received from the Pacific island countries during the period 1996-2008. It was 26% among AFP cases from Brunei Darussalam. VIDRL has performed parallel testing of AFP specimens and isolates from an aseptic meningitis study from the Institute for Medical Research Malaysia (IMR). The IMR agreed to send all AFP samples to the VIDRL beginning in August 2007. During the period 2002-2008, the NPEV rate from Papua New Guinea samples was 30%.

2.2.6.2 China

Poliovirus isolation was performed in 31 provincial laboratories in China, and China CDC (RRL) will perform ITD for poliovirus isolates by PCR followed by sequencing. Since isolation rates of poliovirus and NPEV from AFP cases during the period 1998-May 2008 have decreased, the number of poliovirus isolates sent to RRL during the period 1999-2008 was also decreased. All 31 provincial laboratories in China passed proficiency testing with a 100% score during the period 2005-2007. Cell sensitivity testing also has been implemented in 31 provincial network laboratories. China CDC has arranged regular training and workshops for provincial laboratories. Problems for the network laboratory were (1) viral isolation rates of polioviruses and NPEVs are relatively low; (2) there is a frequent turnover of laboratory staff; (3) some equipment must be updated; (4) the transportation of samples and isolates remains challenging; and (5) some provincial laboratories have low cell sensitivities.

2.2.6.3 Japan

The NIID, Tokyo, has played a role of being the global specialized laboratory (GSL) for the GPLN, the RRL for Viet Nam, Mongolia, and the Republic of Korea and the NPL for Cambodia, the Lao People's Democratic Republic, and Japan.

From Cambodian AFP samples, the NIID isolated four type 3 VDPV strains (two in 2005 and two in 2006). From the Lao People's Democratic Republic's AFP samples, two type 2 VDPV strains were isolated in 2004. Decreased numbers of AFP samples were referred from Cambodia and the Lao People's Democratic Republic during the last few years. However, the nonpolio virus (NPV) isolation rates have been maintained at 20%-30% during this period.

The NIID has applied its own PCR-restriction fragment length polymorphism (PCR-RFLP) method as well as ELISA, monoclonal antibody ITD, and the entire VP1 sequencing for ITD of poliovirus strains. The NIID already introduced the new algorithm for virus isolation and ITD and also will revise the SOP.

The NIID has an extensive surveillance system for enterovirus (aseptic meningitis, hand foot mouth disease (HFMD), herpangina, and acute encephalitis), and the results are published in the Infectious Agents Surveillance Report. Local prefectural laboratories are responsible for virus isolations. The NIID has operated annual training courses for the GPLN with support from the Japan International Cooperation Agency (JICA). The NIID also provided diagnostic support for HFMD outbreaks in China, Viet Nam, and Mongolia in 2008.
2.2.6.4 Hong Kong (China)

The Virology Division, Center for Health Protection, was designated as the NPL in 1992 and ITD has been conducted since 2002. This laboratory also receives AFP samples from Macao (China). During the period 2001-2007, 108 AFP cases from Hong Kong (China) and eight cases from Macao (China) have been investigated and no poliovirus has been detected. Three VDPVs were detected from the ITD of polioviruses. The laboratory has a problem of cell sensitivity with L20B cells, but the problem (possibly the CO2 incubator) recently was solved.

2.2.6.5 New Zealand

New Zealand switched from oral poliovirus vaccine (OPV) to inactivated poliovirus vaccine (IPV) in February 2002. The laboratory was involved with research into the discontinuation of OPV after polio eradication. The study concluded that the circulation of OPV strains does not persist for an extended period in a developed country with a temperate climate after the switch is made. OPV strains declined very quickly and significantly after the switch. Data from paediatric inpatient surveillance, enterovirus surveillance, and AFP surveillance indicated that the capability for OPV to establish sustained person-to-person transmission in New Zealand is limited. Sequencing data for environmental isolates collected six, nine, and 12 months after the switch indicated possible importation.

2.2.6.6 Singapore

AFP surveillance was established in 1995 to test samples from all patients with diseases that could lead to AFP regardless of whether AFP is present. This laboratory has been accredited since 1998 and tested 160 AFP samples during the period 2002-2007. A total of 3506 samples from various sources were tested for enterovirus during this period. NPEV isolation rates from stool samples remained >10% ranging from 10%-48% during this period.

This laboratory has been accredited for ITD function since 2003 and performed ITD for 12 poliovirus isolates using PCR and ELISA methods. All strains were Sabin-like strains except one type 1 wild poliovirus that was isolated in 2006. Since the new algorithm was implemented on 10 April 2008, only one poliovirus was isolated in May 2008.

2.2.6.7 Mongolia

A total of 2137 samples were tested for poliovirus during the period 2002-2008. Among those samples, 2.9% were from AFP cases, 2.8% from AFP contacts, 66.4% from healthy children, and 10.8% from children with other diseases. During this period, 33.8% of samples were from Ulaanbaatar, 66.1 from rural areas. Seven aimags, or provinces, had no AFP cases. All 66 polio isolates were sent to the RRL in the NIID, Tokyo. Cell sensitivity testing results showed a decrease in cell sensitivity in both L20B and RD cells. The reasons for the titre decrease should be sought.

During the period May-June 2008, 2361 cases of HFMD were registered: 48.3% in Ulaan Baatar and 51.7% in the provinces. About 245 samples were tested using Codehop PCR. EV71 strains were detected from 102 cases (41.65%) and CA16 strains from six cases (2.4%).

Special attention should be given to high risk populations of unregistered children and mobile and street children. This laboratory also will re-establish environmental surveillance.
2.2.6.8 Malaysia

Wild poliovirus has not been detected since three cases of wild poliovirus importation in 1992. This laboratory was designated as an NPL in 1993 and fully accredited in 1998. Although NPEV rates have been kept low during the period 2005-2007, 87 poliovirus strains were isolated during the period 2002-2008. All of these isolates were sent to the VIDRL, Australia, and confirmed as Sabin-like strains. The shipment of samples from this laboratory to the RRL has been processed by a qualified person who attended a relevant course. There have been eight to nine shipments to the RRL each year during the period 2002-2007.

2.2.6.9 The Philippines

This laboratory was designated as an NPL in 1991, and 5931 specimens from 2990 cases were tested during the period 2002-2008. Poliovirus strains were detected from 56 samples and NPEV from 308 cases. To implement the new algorithm from April 2008, this laboratory requested an office order to work during the weekends and scheduled inoculation was planned to avoid laboratory work on Sundays. Since the implementation of the new algorithm, 291 specimens from 147 cases were examined. Sixteen samples showed CPE or cell degeneration in RD1 cells and four samples showed CPE or cell degeneration in RD2 cells but were confirmed negative on neutralization test. It took an average of 11.4 days from the receipt of samples to obtain the virus isolation results. Some concerns about the new algorithm include slowly growing NPEV, some RD1 that shows CPE that turns out to be negative in RD2 and/or NT, working during the weekends, and shipping costs to RRL.

2.2.6.10 The Republic of Korea

This laboratory operates AFP surveillance among 105 sentinel hospitals and also tests AFP samples. The laboratory introduced web-based reporting and routinely sends newsletters to sentinel sites. It also operates enterovirus sentinel surveillance and newsletters are sent to the 23 participating hospitals bimonthly. For the laboratory diagnosis of enteroviruses, 5'NCR real time PCR and VP1 Codehop PCRs are used as well as cell culture and neutralization tests.

During the period 2002-2007, 32 to 74 AFP samples were tested each year and NPEV isolation rates ranged from 10% to 25.8%. But the rate dropped to 6.5% in 2008. From non-AFP sources, 1222 and 1795 samples were tested in 2006 and 2007, and various enterovirus serotypes were identified each year. Since the shift from OPV in 2004, Sabin strains have not been detected recently. For quality assurance measures, cell sensitivity testing and mycoplasma testing were implemented regularly.

2.2.6.11 Viet Nam

Ha Noi. During the period 2002-2008, 1611 AFP samples were tested and 19.8% were positive for polio or enteroviruses and 28 poliovirus strains were detected. Cell sensitivity testing has been regularly implemented for L20B and RD cells. This laboratory is experiencing a serious problem with shipping isolates to the RRL in Japan. Two polio isolates were not sent to NIID in 2007 and only confirmed as Sabin-like strains in the NPL in Ha Noi by the PCR-RFLP.

Ho Chi Minh City. During the period 2002-2008, 2319 AFP samples were tested and 323 (13.9%) were positive for enteroviruses and 42 poliovirus strains were detected. Cell sensitivity testing has been performed regularly.
2.2.7 VDPV surveillance in China

Dr Yong Zhang made a presentation about VDPV surveillance in China. The extensive VDPV surveillance was initiated in 2003 and, recently, paralytic polio cases due to VDPV have been recognized in either sporadic or outbreak cases in China. For identification of VDPVs, PCR-RFLP, ELISA, and VP1 gene sequencing methods have been used. VDPVs have been found among children with zero dose, incompletely immunized children, or in a region of low oral poliovirus vaccine (OPV) coverage. In 2004, cVDPVs were detected in Guizhou province, highlighting the growing risk of circulating cVDPVs in China because of the increasing gaps in immunity. In 2005, one iVDPV case each of type II and type III was detected in Anhui provinces, implying a serious challenge to polio-free status in China. During the period 1996-2004, eleven aVDPV strains were detected in Shandong (1997), Guizhou (one in 1997 and one in 2004), Yunnan (one in 1997), Gansu (one in 1999), Shanghai (three in 2000), Sichuan (one in 2001 and one in 2002), and Anhui (one in 2002). During the period 2005-2008, 14 aVDPVs were detected in Guangxi (eight in 2006 and one in 2007), Shanghai (one in 2006), Shandong (three in 2007), and Shanxi (one in 2007).

2.2.8 Updates on laboratory containment of wild poliovirus

Dr Sigrun Roesel gave an overview of global and regional updates on the laboratory containment of wild poliovirus. Globally, over 200,000 laboratories were surveyed and about 600 institutions retain wild poliovirus to date. In the Western Pacific Region, only four countries (106 institutions) have wild poliovirus infectious and potentially infectious materials. Phase I containment has been completed in the Western Pacific Region except in China and Japan. Two countries were expected to complete phase I containment this year.

Main issues facing Polio laboratories from Day One:

<table>
<thead>
<tr>
<th>Country</th>
<th>Main issues</th>
</tr>
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<tbody>
<tr>
<td>Japan</td>
<td>Quality control/quality assurance (QC/QA) of molecular ITD and sequencing. Decline in the isolation of polioviruses. Transportation of samples and reagents.</td>
</tr>
<tr>
<td>China</td>
<td>Viral isolation rates of polioviruses and NPEVs are relatively low; laboratory staff turnover, new staff needs training. Some equipment needs updating. Transportation faces new challenges. Some provincial laboratories have low cell sensitivities. Requested new NIBSC Sabin strains in order to set up the cell sensitivity test again (urgent) and ITD regents, especially ELISA ITD reagents (urgent). Provide training opportunities for laboratory staff, especially new staff.</td>
</tr>
<tr>
<td>Hong Kong (China)</td>
<td>Obtaining clinical samples from AFP cases to meet requirements. Timely differentiation of wild and vaccine derived viruses. Determining immunity after switching to IPV.</td>
</tr>
<tr>
<td>Singapore</td>
<td>Concerns with the new algorithm: 1) If NT is performed to obtain monotypes from mixtures for ELISA only after PCR, it may not be possible to report ITD results on AFP cases within seven days; 2) molecular equipment in separate service lab. Access to equipment and facility subject to service demands; 3) with the old ITD algorithm, could perform either ELISA first or PCR. With the new ITD algorithm, it’s PCR first then ELISA. PCR can be a bottleneck; and 4) ITD by PCR and sequencing imposes a greater need on access to molecular equipment. Routine service demands on molecular facility may affect timeliness of carrying out poliovirus ITD. Challenges: 1) Lack of ELISA reagents. No more PV3 NSL control for use; 2) Development of VP1 sequencing as alternative--validated; and 3) Lack of standardization and SOP. Requests: 1) How will PT for polio culture be conducted, reported, and assessed? When implemented? 2) No ELISA reagents. Any more PT? Drop ELISA? Up to individual lab to decide? Sequencing as alternative? 3) Provision of detailed standardized protocol for VP1 sequencing for ITD from WHO. PT for sequencing? 4) If no sequencing PT, what will accreditation criteria be? 5) WHO funding for shipment to RRL, GSL; and 6) Provision of primers and reagents from WHO for sequencing for PCR.</td>
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<tr>
<td>Korea, The Republic of</td>
<td>Improve AFP surveillance and specimen transportation from local regions. Full introduction of tube culture.</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Turnaround time is 14 days instead of 28 days. Contamination and ageing of cells. Early confirmation of polioviruses PCR for isolates in L20B. Restricts use of NT. Low NPEV isolation rate: cell sensitivity (?), extra cell line. Training for new staff, PCR/new algorithm.</td>
</tr>
<tr>
<td>Mongolia</td>
<td>Regular servicing of equipment and satisfaction with some equipment: Biosafety cabinet class II, -70°C Freezer etc. Adequate staffing. Training.</td>
</tr>
<tr>
<td>Philippines</td>
<td>Slow growing NPEV. Some RD1 exhibiting “CPE” turns out negative in RD2 and/or ENT. Personnel need to report on weekends. SOP and worksheets have not been changed. Cost of shipments to RRL.</td>
</tr>
<tr>
<td>Viet Nam/Ha Noi</td>
<td>RT-PCR sequencing method to confirm wild or Sabin-like or VDPV. Staff in the laboratory: 03 staff (2 new staff with 1-3 experiment years). Shipping polio isolates to RRL.</td>
</tr>
<tr>
<td>Viet Nam/Ho Chi Minh City</td>
<td>Cost of shipments to RRL.</td>
</tr>
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</table>
2.3 Strengthening of Measles/Rubella Laboratory Network

2.3.1 New requirements for global measles laboratory network and WHO genotype database

Mr David Featherstone presented new requirements for the global LabNet and WHO genotype database. Molecular capacity such as PCR and sequencing equipment have become accessible to more laboratories and oral fluid samples allow detection of measles and rubella genome weeks after onset. Genetic information in combination with epidemiological data can allow the mapping of transmission pathways, identify a possible source of a virus, and differentiate indigenous or imported and vaccine-related or wild type infection. It also can provide an indicator for quality of surveillance systems and can be useful in diagnosing in the first few days after the onset of a rash.

However, laboratories with genetic data are not aware of the importance of sharing with the surveillance programme. Data are released sometimes months or years after sample collection. Countries close to eliminating the virus should know as soon as possible whether cases detected are the result of an imported or an indigenous virus. In this regard, WHO measles/rubella virus genotype database in an Excel format was created in 2006 and 2823 measles virus strains (18 genotypes) and 194 rubella virus strains (11 genotypes) during the period 1979-2008 were included. Among six WHO regions, the European Region has submitted data for 1200 measles strains and the Western Pacific Region submitted data for about 600 strains from four countries. H1 genotype was the prevalent genotype in the Western Pacific Region during the period 1995-2008, and most H1 strains were from China. H2, G3, D9, D5, D4, and A strains also were put into the database from the Western Pacific Region during this period. However, there were only two rubella strains put into the WHO database from the WPR: The Republic of Korea (1996) and Malaysia (2001).

Timely contributions to the WHO genotype database would be significant since reporting the timeliness of genotype data is a criterion of accreditation assessment for the WHO measles laboratories. The results of virus detection and genotyping could be completed within two months of the receipt of specimens. Data should be reported to WHO monthly for >80% of samples appropriate for genetic analysis.

The new checklist for the national measles laboratory also emphasizes that there should be separate areas for nucleic acid extraction, amplification, and product detection and a dedicated "clean" area for the preparation of reagents.

Regular confirmatory testing should be performed to ensure performance quality of the national laboratories and to detect problems in a timely manner.

As a quality control measure, validated assay should be used for IgM detection and in-house control for EIA should be included. Monitoring of in-house and kit controls should be presented as a graphic display and temperatures of temperature-sensitive equipment such as incubators, freezers, and refrigerators should be monitored daily. For laboratories performing PCR, appropriate controls should be used for each PCR run.

2.3.2 Measles PT updates

Dr Jennie Leydon presented measles proficiency testing updates. The panel is composed of 20 samples, including measles IgM positive samples, rubella IgM positive samples, parvovirus IgM positive samples, dengue IgM positive samples, and measles/rubella IgM negative samples. These samples are screened for HIV, hepatitis B vaccine (virus)
(HBV), and human chorionic gonadotropin (HCG). Pretesting of samples is performed using different kits of measles (Dade Behring Enzygnost and Chemicon Light Diagnostics) and rubella (Beckman Access anti-rubella virus IgM capture ChLEIA and DiaSorin, Dade Behring Enzygnost). These panels have been sent to 166 laboratories of the global measles and rubella laboratory network. In the Western Pacific Region, Dade Behring, Denka Seiken, Virgo, Serion, Euroimmun, Wampole, and Biorad kits were used for measles. Dade Behring, Denka Seiken, Biorad, Serion, Adaltis, and Wampole kits were used for rubella. To replenish the specimen stocks for measles PTs, donations of IgM positive sera for measles, rubella, dengue, and parvovirus from countries are recommended.

2.3.3 Updates on alternative sampling techniques and new developments

DBS and oral fluid have been validated comprehensively, and these alternative samples would be useful when serum collection or maintaining cold chain is challenging. Sensitivity and specificity of DBS and oral fluid compared to serum samples were 99.4%-100% and 91.4%-100% (DBS) and 96.1%-99.2% and 83.5%-100% (oral fluid). Stability of oral fluid and DBS samples for IgM testing was evaluated. Oral fluid samples were stable for up to a week at ambient temperatures and DBS samples were stable for up to several months at room temperature.

The advantages of these alternative sampling methods would be: (1) almost equivalent sensitivities to serum for measles and rubella IgM detection; (2) temperature stability; (3) RNA detection can be extended from days to weeks with oral fluid; (4) potential reduction of transport cost (without cold chain); and (5) greater patient acceptance. Challenges for alternative sampling methods would be: (1) critically important training for collection, especially DBS; (2) supplies of DBS and oral fluid collection devices should be supplied to health centres; and (3) QA programme has not been established.

2.3.4 Updates of measles molecular epidemiology

Dr Paul Rota from the CDC presented updates of measles molecular epidemiology. Molecular techniques must be used in conjunction with standard epidemiologic techniques and can provide a valuable tool to: (1) monitor the transmission of measles virus during and after outbreaks; (2) differentiate a repeated transmission of endemic virus from a new, imported source of virus; (3) classify unusual or severe cases; and (4) confirm suspected vaccine reactions. There is a need to compare sequences in addition to genotypes.

In the United States of America, molecular epidemiological data collected during the period 1989-2006 have documented the interruption of transmission of D3 and multiple genotypes from importations that have been detected since 1994. Emerging challenges for the laboratory would be waning immunity in the absence of natural boosting and secondary vaccine failure. Secondary failure cases pose diagnostic challenges and are likely to be missed unless investigation identifies rash illness among contacts during and after outbreaks. Measles cases with secondary immune responses can exhibit a milder disease, either IgM negative or low reactive IgM, IgG positive and RT-PCR positive results.

Lessons learnt from molecular epidemiology of measles virus would be: (1) that outbreaks anywhere mean outbreaks everywhere because of the rapid global travel of measles lineages (recent examples include Ukraine D6, Romania D4, Kenya/Somalia B3, and Switzerland/Japan D5 strains; (2) that transmission can occur anywhere such as airports and amusement parks; (3) that vaccination programmes frequently interrupt the transmission of measles lineages but reintroduction is a problem. A switch in genotypes and a decrease in complexity within genotypes can occur. Predominant genotypes have been changed C2-D6-
D7-D4/D6-D6 in Europe and D3/D5-D9/H1-D5 in Japan; and (4) that all measles vaccines are genotype A and antigenic change does not change vaccine efficacy.

Current wild type viruses are recognized by serum from vaccines. Measles vaccine has been effective globally despite different endemic genotypes. However, molecular studies alone cannot differentiate between continuous circulation and multiple introductions from the same source. Measles virus databases in the United States CDC and HPA laboratories are linked. Laboratories can submit genotype information with or without an accession number to the measles/rubella database at WHO to capture additional epidemiologic and virological information and link to GenBank accession numbers.

2.3.5 New developments of the Western Pacific Region measles/rubella laboratory network

Dr Youngmee Jee presented new developments in the Western Pacific Region measles/rubella network. This laboratory network is composed of four global or regional reference laboratories, 16 national laboratories, and 31 subnational plus 331 prefectural laboratories in China. Confirmatory testing of samples was performed for 3.1% of measles samples and 2.7% of rubella samples in 2007. Some recommendations from the 2004 measles laboratory network meeting have not been implemented and should be reviewed thoroughly.

Genotypes of measles virus strains circulating in the Western Pacific Region during the period 2006-2008 were H1, G3, D9, D8, D5, and D4. New checklists for national measles laboratories (NMLs) that emphasize the timely reporting of virus detection and genotyping results will be used from the 2008 accreditation visit. The new measles/rubella laboratory reporting form will be used and the deadline for monthly reporting is the tenth day of every month.

Laboratories with the ability to perform virus culture and molecular detection are encouraged to conduct virus isolation and molecular detection of measles and rubella viruses.

IgM detection in serum samples remains the gold standard for confirmation of measles and ELISA using dried blood spots (DBS) can be applied among countries with moderate to high measles incidence with some difficulties in sample transport.

2.3.6 Discussions on the new reporting format and data management

A new format to report measles and rubella laboratory data to the WPRO was distributed in January 2008 and the form for recording data was explained in detail. Monthly summary data was added to the line lists.

2.3.7 Country reports

2.3.7.1 Macao (China)

Serosurvey data for measles in Macao (China) were presented and no IgM positive case was found during the period 2002-2005. During the period 2006-2007, four and two IgM positive cases were detected, respectively. The measles IgG positive rate under 20 years was lower than 70% and most IgM positive cases were related to vaccination.
2.3.7.2 Mongolia

The NML in the National Center for Communicable Diseases collects all specimens from suspected measles cases from all over the country. This laboratory can perform virus isolation and an immunofluorescent assay (IFA) test in addition to ELISA. During the period 2002-2003, 12 measles virus strains were isolated (six in 2006 and six in 2003), and the sequence analysis showed that all isolates belonged to genotype H1. This laboratory tested 2030 and 203 samples for measles in 2007 and 2008, respectively, and 13 and 12, respectively, showed positive for measles. For rubella, 1567 and 135 samples were tested for rubella IgM in 2006 and 2007, respectively, and 964 and 23 samples, respectively, showed positive for rubella. This laboratory was planning to introduce Vero/signalling lymphocyte activation molecule (SLAM) cell culture and molecular detection for measles and rubella.

2.3.7.3 Cambodia

The immunology unit at the National Institute of Public Health (NIPH) was designated as an NML. Since there are many rubella positive cases, this laboratory tests for rubella IgM first and then tests rubella negative samples for measles IgM. This laboratory began to use DBS along with serum samples from 2006. In 2007, 152 DBS samples and 910 serum samples were tested. Among serum samples, eight were positive for measles and 174 for rubella (19.1%). Among DBS samples, 13 showed positive for rubella (8.5%) and two showed positive for measles. In 2008 (as of May), 1383 serum samples and 319 DBS samples were tested. Among serum samples, four showed positive for measles and 341 (24.6%) showed positive for rubella. Among DBS samples, 13 (4.0%) showed positive for rubella, but measles was not detected. About 41% of laboratory results were sent to the National Immunization Programme (NIP) within a week and 71% of the results were sent within two weeks. The quality of samples must be improved.

For confirmatory testing, 50 samples from 2007 were sent to Hong Kong (China) in March 2008. Although the concordance rate for measles testing results was about 90%, all measles positive cases were negative in Hong Kong (China). Therefore, it was recommended to send additional confirmatory samples during the second half of 2009.

2.3.7.4 The Lao People's Democratic Republic

The Virology section at the National Center for Laboratory and Epidemiology (NCLE) serves as the NML in the Lao People's Democratic Republic. Samples from 2008 show that rubella IgM testing has been implemented regularly. There is no capability for molecular testing, virus genotyping and IFA in the NCLE. During the period 2002-2004, 372 samples were tested for measles and 268 samples were tested for rubella. Among the samples tested, 270 (72.6%) showed positive for measles and 16 (5.97%) showed positive for rubella. Measles and rubella IgM testing was not conducted during the period 2005-2006. In 2007, among 133 samples tested for measles, 109 showed positive for measles. In 2008, 94 samples were tested and none showed positive. But 32 rubella positives were detected. Before 2008, the NCLE received only five to ten samples from each outbreak. From 2008, the NCLE asked the provinces to collect samples from all sporadic cases as well as outbreak cases.

2.3.7.5 The Philippines

The Virology Laboratory in the Research Institute for Tropical Medicine (RITM) has been designated the NML since 2001. For the quality control (QC) of the laboratory, internal and external (in house) controls are used and a graphic display of QC values has been
available since 2006. During the period 2004-2008, 1449 samples were received and 51.9% of the results (n=752) were reported within seven days. Among those samples, 509 (32.9%) showed positive for measles and 414 (32.2%) showed positive for rubella.

2.3.7.6 Papua New Guinea

The serology unit at the Central Public Health Laboratory is designated as the NML. It collaborates closely with the VIDRL. Dr Michaela Riddell from the VIDRL, Melbourne, trained nine staff members for DBS in August 2007. During the period 2003-2008, 86 samples were tested and 12 measles cases and eight rubella cases were detected. There were three measles false positive cases in 2008. There is a need to improve regular reporting to the national surveillance unit and also to the WPRO.

2.3.7.7 Fiji

The laboratory in the Mataika House is designated as the NML. During the period 2004-2008, 166 samples were tested for measles and 54 for rubella. Seventeen samples, including 13 positives from 2006 outbreaks, showed positive for measles and eight showed positive for rubella. National data for measles reporting comes from three different systems: the Patient Information System (PATIS), with a computer network in Fiji and Samoa recording hospital admissions and outpatient visits; the National Notifiable Disease Surveillance System (NNDSS); and the Fiji Centre for Communicable Disease Control (FCCDC). The reported numbers from those three systems were different during the period 2004-2008: 47 for PATIS, 218 for NNDSS, and 166 for FCCDC. The Ministry of Health State Unit publishes cumulative updates quarterly to public health institutions and, through the FCCDC, compiles reports for collaborating partners. FCCDC plans to strengthen the surveillance unit to enable timely and accessible publications of laboratory and clinical data.

2.3.7.8 Viet Nam

Ha Noi. The National Institute of Hygiene and Epidemiology has been designated as the NML for the northern part of Viet Nam. ELISA kits used for measles and rubella during the period 2004-2008 were Dade Behring (WHO-provided), Denka Seiken, Biorad, and Bioquant (NIHE-provided). For proficiency testing for measles and rubella, NIHE used the Denka Seiken kit for measles and Dade Behring for rubella in 2007 and Biorad kits for both measles and rubella. During the period 2004-2008, 5925 samples were tested for measles and 434 samples (7.3%) showed positive. For rubella, 5492 samples were tested and 3105 samples (56.5%) showed positive. Ten measles virus isolates were analysed in 2006 (6) and 2008 (4). Laboratory data was shared with the national EPI surveillance and laboratory staff who attend the EPI quarterly review meetings.

Ho Chi Minh City. The Pasteur Institute in Ho Chi Minh City is designated as the NML for southern Viet Nam. Dade Behring, Biorad, and DSL were used for measles and rubella IgM testing during the period 2004-2008. A total of 7964 samples were tested for measles and rubella. Nineteen samples (0.24%) showed positive for measles and 4958 samples (62.3%) showed positive for rubella. For confirmatory testing, 16 samples were sent to Hong Kong (China) in 2006. More samples should be confirmed by confirmatory testing in the RRL. Considering the high positive rate for rubella, it would be beneficial to test the samples first for rubella IgM followed by measles IgM.
2.3.7.9 Malaysia

The Serology Laboratory in the National Public Health Laboratory (NPHL) is designated as the NML. The NPHL has moved to a new location in Selangor. The laboratory is well equipped with separate serology, virus isolation, and molecular laboratories. The laboratory can perform virus isolation and molecular detection of measles and rubella viruses. During the period 2004-2008, there were 1307 (17%) measles positive cases among 7664 samples that were confirmed by IgM ELISA. But the positive rates decreased from 36.3% in 2004 to 1.9% in 2008. Also, 1113 rubella cases (26.4%) among 4210 samples were confirmed by IgM ELISA during the sample period. During this period, 680 samples for virus isolation were collected, and measles viruses were isolated from 53 cases (7.9%) and rubella viruses from 24 (3.5%) cases. The measles isolates were sent to the VIDRL, Melbourne, for genotyping and confirmed as D9 genotype.

2.3.7.10 The Republic of Korea

The Division of Respiratory Viruses in the Korea Center for Disease Control and Prevention (KCDC), Seoul, serves as the NML. It performs parvovirus B19 IgM ELISA (Biotrin) and HHV-6 IgM IFA (Biotrin) in addition to measles and rubella testing. During the period 2004-2008, 1612 samples were tested for measles and 362 cases were confirmed by the laboratory. The positives include 290 IgM cases, nine virus isolates, and 63 RT-PCR cases. During the period 2006-2007, 359 measles negative samples were tested for rubella, parvovirus B19, and HHV-6 and 11 rubella positive, 10 parvovirus B19 positive, and 120 HHV6 positives were detected. Sequence analysis of measles virus strains showed that the 2007 outbreaks were caused by H1 strains and one D5 strain that might have been imported from Japan. One D5 strain was also detected from a sub acute sclerosing pan encephalitis (SSPE) patient in 2005.

A quality assurance programme for 17 provincial laboratories and five private diagnostic centres was arranged in 2007 comprising a proficiency testing programme and a confirmatory testing programme. All 17 provincial laboratories used Dade Behring kits while six private diagnostic centres used Radim (2), Euroimmune(1), and DSL (1). There were 10 proficiency test samples in 2007 that showed five measles positives, one rubella positive and four negatives. The 2008 quality assurance panels for provincial laboratories and five diagnostic centres and training for measles virus isolation for provincial laboratories also were planned.

2.3.7.11 Singapore

The Virology Laboratory at Singapore General Hospital is designated as the NML and performs virus isolation, immunofluorescence (IF) and sequencing, and IgM ELISA testing. This laboratory used the Dade Behring ELISA kit for measles and the EIAgen kit for rubella. Since the testing was conducted based on requests from clinicians, not all samples were tested for rubella or measles. This laboratory has participated in the College of American Pathologist (CAP), the Royal College of Pathologists of Australia (RCPA), WHO, and CDC proficiency programmes and fully implemented various quality assurance measures. During the period 2006-2008, 19 samples were sent to the RRL, and genotypes G3 and D9 in 2006, D5 and D9 in 2007, and D9 and D4 in 2008 were detected.

2.3.7.12 New Zealand

The Serology and Virology Laboratory in the Canterbury Health Laboratories in Christchurch is designated as the NML. The Institute of Environmental Science and
Research (ESR) coordinates the operation of the national notifiable disease surveillance database EpiSurv on behalf of the Ministry of Health. The NML performs parvovirus IgM ELISA and measles and rubella ELISA testing and also RT-PCR. Genotypes G3 and D9 recently were detected. Private laboratories also perform measles and rubella ELISA and participate in ISO 92002. However, there is no coordination with these laboratories by the NML.

2.3.7.13 Japan

The National Institute of Infectious Diseases (NIID), Tokyo, has been designated as the GSL and the RRL and contributed to the global measles and rubella laboratory network by characterizing and distributing Vero/SLAM cells to the network laboratories. The NIID also has collaboration projects for measles elimination in China supported the JICA. Since the measles epidemic (D5) and some exported cases to other countries in 2007, Japan has established the national strategies for measles elimination by 2012. From the epidemiological investigation of measles cases, it was noted that 46.5% were not vaccinated. Therefore, a five-year plan for supplemental measles vaccination for 12- and 17-year-olds started in April 2008. Beginning in 2008, all measles cases were to be reported to the national surveillance system. Nine measles reference centres have been established that can perform RT-PCR, sequencing, virus isolation, and IgM ELISA. These centres will provide technical support to the 77 prefecture institutes.

2.3.7.14 Australia

The VIDRL, Melbourne, is designated as the RRL for the Western Pacific Region and receives samples from member states for confirmatory testing. The VIDRL also produces and distributed proficiency test panels for the global measles and rubella laboratory network. The VIDRL uses Dade Behring and Chemicon Light Diagnostics Measles IgM ELISA and Beckman Access, DiaSorin, and Dade Behring Rubella IgM kits. It also tests for parvovirus IgM in rash illness samples. In Australia, Abbott AxSym MEIA, bioMerieux, Vidas, ELFA, Dade Behring EIA, and DiaSorin EIA are used, and laboratories participate in the Royal College of Pathologists of Australasia (RCPA) quality assurance programme. Imported cases of measles because of different genotypes mainly have been reported.

2.3.7.15 Hong Kong (China)

The Virology Division of the Center for Health Protection (Government Virus Unit) was designated and accredited as the NML in 2006. The laboratory uses Microimmune and Dade Behring IgM ELISA kits for measles and also Complement fixation, virus isolation and RT-PCR and sequencing targeting N gene (545 bp). H1 strains have been predominant and D4, D9, and A strains also were detected during the period 2004-2008. For rubella testing, Dade Behring EIAs, and DiaSorin EIAs are used. Also used are the Hemagglutination inhibition test, virus isolation, and RT-PCR targeting E1 gene (103bp) for diagnostic purposes. For quality assurance, the laboratory participates in ISO 15189 as well as WHO accreditation. Since May 2007, this laboratory was re-designated as an RRL for the Western Pacific Region for validation of samples from the NML and molecular surveillance. Since 2007, the laboratory performed the retesting of 137 samples for measles and 124 samples for rubella. The concordance rate varied from 60%-100% for measles and 89%-100% for rubella. H1, D4/D9, and H1 strains were detected from samples referred from Viet Nam, Singapore, and the Lao People's Democratic Republic, respectively.
2.3.7.16 China

The China CDC plays a role for RRL for the Chinese measles laboratory network, which is composed of 31 provincial laboratories and 331 prefectural laboratories. China CDC provides genotyping, quality control, accreditation, and technical support for provincial laboratories. They will perform measles virus isolation and identification by PCR, ICA, RFLP, and real time PCR and quality control, accreditation, and technical support for prefectural laboratories. Prefectural laboratories perform most of the primary serology for suspected measles patients.

The measles laboratory network in China collected 60 173 samples for measles testing and 83.4% (n=50 197) were tested. In 2007, about 100 000 measles samples were collected and 34.9 % (n=40 742) showed positive for measles. About 50 000 samples were collected for rubella and 9.1% (n=7073) showed positive. During the period January-May 2008, 35 555 cases were laboratory confirmed for measles among 97 047 cases and 9761 cases were laboratory confirmed for rubella among 89 793 cases.

During the period 1993-2008, 1168 measles strains were isolated and H1 strains were predominantly detected. Among three sub-genotypes (H1a, H1b, H1c) detected during this period, only H1a strains were discovered during the period 2006-2008. For quality control measures, proficiency testing and reconfirmation tests, onsite reviews, and training and workshops have been implemented annually. All kits used in China including Dade Behring, Virion Serion (indirect IgG), and three local kits (Haitai, Kerunda, and Beire) were evaluated. Calibration of micropipettes and maintenance of biosafety cabinets were performed regularly and all laboratories conducted temperature monitoring.

The Chinese CDC also established the Plaque Reduction Neutralization Test (PRNT) as a gold standard method for IgG detection and to evaluate sensitivity and specificity of measles IgG kits. For shipping specimens to the Chinese CDC (RRL), laboratories use the UN No 3373 class B packaging system.

2.3.8 Rubella virology surveillance: Worldview of viruses of different genotypes

Dr Joe Icenogle presented an overview of worldwide virological surveillance for rubella. A 739 bp region of the E1 region has been standardized as the molecular window for genotyping of rubella viruses. There are nine recognized genotypes (1B, 1C, 1D, 1E, 1F, 1G, 2A, 2B, 2C) and four provisional genotypes (1a, 1h, 1i, 1j). For rubella virus isolation, a vigorous throat swab on the day of rash onset (or no more than five days after a rash) would be the ideal specimen. For CRS cases, throat or nasal swabs are good sources of the virus for up to six months after birth. Among different genotypes, 1E and 1G are widely distributed and 2B now also is widely distributed; 1a, 1C, 1F, 2C, 1B, 1h, 1j, 1D are geographically restricted.

2.3.9 Current status of rubella in Japan

Dr Katsuhiro Komase presented the current status of rubella in Japan. During the period 1999-2008, 17 767 rubella cases and 16 CRS cases were reported. Rubella epidemics occurred every five to six years. The second dose of rubella vaccines was introduced in 2006, and all rubella cases were reported beginning in January 2008. Together with a measles elimination strategy, supplementary vaccinations of rubella also were provided to 12-13 year olds and 17-18 year olds for five years. Recent rubella strains identified in 2007 belonged to genotype 2B.
2.3.10 Laboratory surveillance for rubella virus in China, 1999-2008

National surveillance for rubella was established in 2005 and CRS surveillance in selected cities was initiated in July 2007. Surveys on rubella antibody levels among women of childbearing age were conducted in 2006. These surveys showed that the total negative rate among those of childbearing age ranged from 5%-22%, showing that >10% of this population had risk of infection. This data provided the scientific evidence for adopting a rubella immunization strategy in China.

This laboratory performed virological characterizations of wild type rubella viruses isolated in China during the period 1999-2008. Rubella RNA was detected with primers targeting 185bp of E1 region and the 730bp region subsequently was amplified and sequenced for genotyping. During this period, 1F, 1E, 2A, and 2B strains (n=115) were detected from 13 provinces and 1E strains were predominantly detected.

Main issues facing Measles laboratories from Day Two:

<table>
<thead>
<tr>
<th>Country</th>
<th>Main issues</th>
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<tbody>
<tr>
<td>Japan</td>
<td>Measles laboratory. Network is under construction.</td>
</tr>
<tr>
<td>China</td>
<td>Need real time PCR instrument. Need annual training course for new provincial staff. Improve measles database, combine EPI data with lab data. Need more staff in NML. Kits quality control.</td>
</tr>
<tr>
<td>Hong Kong (China)</td>
<td>Too much information to be filled in the monthly measles/rubella form. Impractical to test all cases with fever and rash for measles and rubella. Difficult to obtain blood samples from sporadic cases in the private sector. Difficult to find rubella PCR/culture positive specimens because the number of cases is low and the patients usually present late.</td>
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<tr>
<td>Singapore</td>
<td>Test is performed based on clinician’s request, paid by patients. WHO algorithm for measles and rubella testing is not followed because there is no funding to support additional testing. WHO requests reporting result within seven days of the following month. Measles culture is observed for 14 days in our lab. If a sample is cultured at the end of the month, results will be ready in the middle of the following month. This has been feedback to WHO, and it is agreed in such cases that the report will be completed after the culture result is available. Results obtained in our lab were not comprehensive. A few other labs also performed measles and rubella testing. Funding of samples sent to RRL. Little clinical and epidemiological information is available, making it difficult to supply this information to WHO. Determination of true positive and negative IgM results, different kit has different performance, no kit is 100% sensitive and specific.</td>
</tr>
</tbody>
</table>
| The Republic of Korea | Need to secure sufficient supply of measles and rubella IgM positive sera for panels.  
Need to train new staffs of KCDC.  
Need to train staff in PHERIs for MV isolation. |
| Malaysia | Measles elimination goals and programme awareness at all levels, including private sector health care service providers, to ensure rapid response to any case, including outbreaks.  
Case reporting and the need to immediately notify local public health authorities.  
Increased staff turnover; re-training needed.  
Investment in Dangerous Goods Course for staff because shipment by world courier is expensive and limits shipments to once a year |
| Mongolia | In order to strengthen epidemiological and laboratory surveillances, it necessary to build up laboratory capacity at the Regional Diagnostic and Treatment Centers.  
Introduce Vero-SLAM cell culture and molecular biological techniques at the national measles laboratory.  
Train health professionals on rapid diagnostic methods to allow confirmation of clinical measles cases and to prepare laboratory technologist abroad. |
| The Philippines | Three versions of the Measles Case Reporting Form were in circulation.  
Data quality of case investigation forms.  
Delayed referral of samples to the NRL.  
Delayed delivery of measles and rubella kits. |
| Papua New Guinea | Late reporting and investigation of suspected cases.  
Delay in sample collection and transport; haemolysed or infected.  
Education on more use of DBS.  
Reagent outdates and stock outs.  
Staff issues.  
Refresher training for laboratory staff.  
Dedicated staff for surveillance work. |
| Pacific Island Countries and areas (Fiji) | Prevailing weaknesses in the current lab and lab surveillance systems and processes.  
Resource limitations.  
Staff turnovers.  
Continue anti-measles and anti-rubella reagent procurement support.  
Staff training.  
WHO external assessment and then develop a framework to support establishment of NML status. |
Cambodia
Lack of internal quality control (IQC) samples for measles and rubella tests.
Lack of IQC DBS control samples.
Need to improved the quality of samples collection.
Having times increased the number of samples two and three times, better management and improved human and financial resources will be required.
Inadequate amount of staff to fulfil all laboratory demands.
Staff needs more technical training.
Staff needs more data management skills and training.
Provincial and district staff require more training about field collection of blood specimens
Insufficient number of freezers and refrigerators.
Additional air conditioner is required to maintain temperature outside the freezer area.
There is no generator backup for power outages.
Longer term technical assistance.

Viet Nam/Hanoi
Update training for 02 new staff of measles laboratory.
Dade Behring kits should be delivered annually in December.
01 Freezer (-20°C).
02 biomedical refrigerators.
01 CO₂ Incubator.

Viet Nam/Ho Chi Minh City

2.4 Development of the Western Pacific Regional Japanese Encephalitis Laboratory Network

2.4.1 Status of JE control in the Western Pacific Region

Dr Manju Rani presented the status of JE control in the Western Pacific Region, where 11 countries were considered to be at risk for JE infection: China, Viet Nam, Cambodia, the Lao People's Democratic Republic, Papua New Guinea, the Philippines, Malaysia, Australia, Brunei, the Republic of Korea and Japan. Japan and the Republic of Korea controlled JE with the full implementation of JE vaccination. Surveillance efforts recently began for countries with possible risks. Dr Rani presented two types of surveillance systems: nationwide surveillance and sentinel surveillance and described opportunities and challenges in combining meningitis (Hib, Pneumo, and Meningitis) and JE surveillance as meningoencephalitis surveillance. She also explained ongoing activities of sentinel surveillance sites in the Western Pacific Region.

2.4.2 JE surveillance: progress, issues, and challenges

Dr Asheena Khalakdina presented the status of global JE control and PATH's JE project. About 50,000 cases and 10,000 deaths are reported annually to WHO, but these numbers were recognized to be underestimated. Children from to 15 years old were affected mainly in rural areas, but people of any age can be infected. Adult infection most often occurs in areas where the disease is newly introduced. JE laboratory-based surveillance would be essential to estimate and quantify the national and subnational burden of disease, to characterize the epidemiology, especially in geographical areas and in populations at high
risk, for strategically targeting control efforts and to establish baseline incidence of the disease in order to monitor the impact of an immunization or control programme. Surveillance activities have been intensified in several countries in the South-East Asia Region (India, Indonesia, and Nepal) and the Western Pacific Region (Cambodia, China, the Philippines, and Viet Nam). Integrated surveillance for acute meningitis and encephalitis has been performed in some countries, and laboratory testing for JE and other vaccine preventable pathogens (Hib, *Str. Pneumo, N. Meningitidis*) using CSF specimens has been conducted.

Dr Khalakdina also presented information about JE vaccines that were available, including inactivated mouse brain-derived vaccine and live attenuated SA14-14-2 vaccine. In the WPR, there is no JE vaccination programme in Cambodia, the Lao People's Democratic Republic, and the Philippines. China, Japan, the Republic of Korea, and Viet Nam have national JE vaccination programmes in place.

2.4.3 Role of the laboratory for JE surveillance

Mr David Featherstone of WHO Headquarters made a presentation about the role of the laboratory for JE surveillance. The recommended case definitions for acute encephalitis syndrome surveillance were:

1. A person of any age at any time of year with the acute onset of fever and at least one of (a) change in mental status, including symptoms such as confusion, disorientation, coma, or inability to talk; and (b) a new onset of seizures, excluding simple febrile seizures.

2. Other early clinical findings may include an increase in irritability, somnolence, or abnormal behaviour greater than that encountered with usual febrile illness.

There are other possible causal agents for acute encephalitis syndrome (AES) such as dengue virus, West Nile virus, Nipah virus, herpes simplex virus (HSV), varicella zoster virus (VZV), measles virus, mumps virus, poliovirus, Enterovirus 71, and Coxsackievirus A.

Because of some cross reactivity with other flaviviruses, there can be a specificity problem with using IgM ELISAs in dengue endemic areas. Antibody response to JE infection can be delayed and there is only 95% positivity 10 days after the onset of illness. CSF is the preferred sample from AES patients. It is recommended to collect two serum samples: a first sample on admission and the second sample at least seven days after or on discharge or death. So far, only a few validated assays are commercially available and further confirmatory testing is needed. Three commercial kits from Panbio, Inbios, and XCyton have been evaluated. The Panbio kit showed slightly lower sensitivity and higher specificity compared to InBios and XCyton. Additional evaluation studies were in progress using surveillance samples from Nepal, Cambodia, and other countries to further establish performance of these assays.

India established JE/AES surveillance using strengths of the National Polio Surveillance (NPSP). The South-East Asia Regional Office has conducted several laboratory training workshops for six countries in its Region. The South-East Asia Regional Office has implemented proficiency testing among 11 network laboratories, receives monthly reporting, and also introduced JE/AES case line listed data collection for the laboratory.
2.4.4 Development of the Western Pacific Regional JE laboratory network

Dr Youngmee Jee presented a regional plan to establish a JE laboratory network in the Western Pacific Region. JE/AES surveillance was introduced in some JE endemic countries: Cambodia, the Philippines, the Lao People’s Democratic Republic, Viet Nam, Papua New Guinea, and China. At the regional level, some countries already have the full technical ability for JE diagnosis while other countries still were building up their laboratory abilities. The biregional JE meeting in 2007 emphasized the importance of strengthening laboratory abilities for JE and initiating the JE laboratory network in the Western Pacific Region.

The main objective of the JE laboratory network would be to develop standards for the laboratory diagnosis of JE and to provide the necessary technical support as JE control evolves in the Region. The JE laboratory network based on a WHO LabNet structure consisting of one GSL, two RRLs, and six to seven national laboratories was to be established in the Region in 2008. The roles of national, regional reference, and global specialized laboratories, and proposed performance criteria for accrediting JE laboratories were described. During the period 2008-2009, national, regional, and global specialized laboratories will be identified and monthly laboratory reporting will be formulated and distributed. Hands-on training and JE proficiency testing panels was to be arranged by 2009.

2.4.5 Country reports on JE situations and laboratory activities

2.4.5.1 China

The JE laboratory in the Department of Vial Encephalitis and Arbovirus, Institute of Viral Disease Control and Prevention, China CDC, has conducted JE diagnosis using IgM capture ELISA in CSF or serum samples, IFA, PRNT, virus isolation, RT-PCR, and real time PCR. Locally produced JE Kits (Beixi and Yueda) used in China have been evaluated and the Beixi kit was found to be more sensitive and specific. Proficiency test panels have been implemented in four provincial and four prefectural laboratories as part of an acute meningoencephalitis syndrome (AMES) project supported by the CDC.

Since 2005, laboratory diagnosis of JE cases has been reported to the national surveillance programme in China. The JE laboratory at the Institute of Viral Disease Control and Prevention was reviewed and evaluated by the WHO expert team in February 2007. A national JE laboratory hands-on training course also was held. Reported JE and dengue cases in China numbered 4331 and 540, respectively, in 2007. JE cases only were detected in the southwest provinces while dengue cases only found were in two southeast provinces.

2.4.5.2 Cambodia

In Cambodia, JE has been recognized for many years as a serious disease but information about its burden has been limited. Some hospital-based studies showed that 20%-30% of encephalitis cases were due to JE. In May 2006, the Cambodian CDC, the National Institute of Public Health (NIPH), the National Immunization Program of the Ministry of Health (NIP/MOH) of Cambodia, PATH, and WHO initiated a hospital-based sentinel JE surveillance project targeting children under 10 years old with suspected meningoencephalitis. Six sentinel sites were selected and CSF and paired sera samples were collected from 572 cases. The NIPH received and analyzed samples using the Panbio JE-Dengue COMBO ELISA kit. Eighty-eight cases (15.3%) showed positive for JE. Of these positive cases, the positive rate of the first serum was 67%, the positive rate of a CSF sample
was 62%, and a first serum and/or a CSF had a positive rate of 87%. The positive rate for the second serum was 13%.

For confirmatory testing, samples during the period May 2006-December 2008 were sent to the WHO global JE laboratory at the CDC. The CDC performed confirmatory testing using CDC ELISA, PRNT, and repeated Panbio ELISA for JE. CSF samples from children under 5 years old that were JE/dengue IgM negative also were tested for bacterial pathogens by PCR. A total of 1195 samples from 451 cases were referred to the CDC and 1008 samples showed concordant results: 61 JE positive and dengue negative cases, 82 dengue positive and JE negative cases, and two JE and dengue positive cases. A total of 183 samples from 101 cases were retested by PRNT and had repeat Panbio ELISA. Six samples (3.3%) were positive for Hib, two samples (1.1%) were positive for N. meningitides, and one sample (0.5%) was positive for *S. pneumoniae*.

2.4.5.3 Japan

The Laboratory of Vector-borne Viruses, NIID, conducts JE IgM capture and IgG ELISA using in-house kits, PRNT/FRNT (focus reduction neutralization test), TaqMan RT-PCR, Conventional nested RT-PCR, and virus isolation using C6/36 and Vero 9013 cells. Provincial laboratories perform HI tests and PRNT. The FRNT technique was transferred to 10 prefectural laboratories during the period 2006-2007. While PRNT takes seven days to obtain results, FRNT only takes three days and many samples can be tested in one 96 well plate. JE HI antibody surveys among pigs and virus isolation from pigs also were performed. The JE virus detected belonged to genotype 1. Since 1992, there have been less than 10 clinical JE cases each year. However, JE viruses are still active among pigs during summers. A new JE vaccine using the Vero cell is being developed and was expected to be licensed by 2009.

The NIID laboratory conducted validation of NIHE, Ha Noi, in-house JE IgM capture ELISA kit, by comparing it with in-house kits from the NIID, Japan. It found a good correlation between NIID, Tokyo, and NIHE, Ha Noi, kits by using 10 serum samples from five JE patients, 16 serum samples from seven dengue patients, eight serum samples and one CSF sample from five other viral encephalitis patients, and one tick-borne encephalitis patient serum sample and three serum samples from healthy donors vaccinated with JE inactivated vaccine.

2.4.5.4 The Lao People's Democratic Republic

The National Center for Laboratory and Epidemiology (NCLE), Mahosot Hospital Welcome Trust Oxford University Tropical Medicine Research Laboratory and three other hospitals have been involved in JE laboratory testing for continual surveillance. Panbio kits are used in both the NCLE and the Mahosot hospital and. In addition, XCyton kit, Hapalyse Dengue JE MP PA kit-Pentax and DBS methods were used in the Mahosot hospital. Among 330 AES patients tested, 13.2% had anti-JEV IgM in CSF samples. A total of 59% of the positive cases were <15 years old and 26% were <5 years old.

2.4.5.5 Malaysia

The Virology Unit at the Institute for Medical Research (IMR) is the main laboratory that receives JE samples, but there also are other laboratories that can perform JE diagnostic testing (National Public Health Laboratory, University Malaya Medical Centre, and Universiti Malaysia Sarawak). In Malaysia, viral encephalitis is a notifiable disease but JE cases cannot be quantified accurately because no specific agent is recorded. For serological
assays, HI and JE IgM capture (since 1991) have been used. Virus isolation and molecular detection can be performed in IMR and other laboratories. As an external quality assurance, IMR participates in the RCPA programme for alphavirus/flavivirus diagnostics. In addition to laboratory based surveillance for JE, hospital-based surveillance was performed in Sarawak during the period 1997-2006. In Sarawak, JE vaccination (nine months, 10 months and boosters 18 months and every three years until 15 years of age) started in 2002. Vaccination coverage in Sarawak was 85.0% in 2007. In peninsula Malaysia and Sabah, vaccinations are performed within a 2-kilometre radius when there is a JE case.

2.4.5.6 Philippines

There has been no clear disease burden study for JE in the Philippines. Sentinel surveillance for meningitis, encephalitis, and meningoencephalitis was initiated in 2008. Five sentinel sites have been designated and CSF and serum samples will be collected from 250 cases (50 cases per site). The objectives of this study were to estimate the proportion of all cases of meningoencephalitis among the population less than 18 years old that are caused by VPD (JE, Hib, N. meningitis, Pneumococcus), to assess the age distribution of JE and bacterial meningitis cases, and to confirm the laboratory diagnosis of JE, Hib, and Pneumococcus from the selected hospitals. The Virology Laboratory at the Research Institute for Tropical Medicine, Manila, will conduct laboratory confirmation using Panbio JE/Dengue Combo IgM ELISA kit (CSF and serum samples). Serum samples will be tested using Panbio JE/Dengue IgM ELISA kits and CSF samples will be tested by Latex agglutination/ bacterial culture at sentinel laboratories followed by bacterial PCR and Panbio JE/Dengue Combo ELISA.

2.4.5.7 Papua New Guinea

The first human JE case was reported in 1997 after outbreaks in Torres Strait in Australia in 1995. It therefore was postulated that the Eastern Province in Papua New Guinea might be a region of JE infection. The Central Public Health Laboratory was not performing JE testing but conducts dengue ELISA testing using serum samples from central and provincial hospitals.

2.4.5.8 The Republic of Korea

JE is classified as Category II National Notifiable Diseases and JE vaccination began in 1968. Fewer than 10 cases were reported annually since 1984. From the sequence analysis of JE viruses detected in the Republic of Korea, strains detected before the 1980s belonged to genotype 3. A new genotype 1 was introduced into the Republic of Korea in 1991. IgM ELISA, IFA, HI, PRNT for serological detection, virus isolation using C6/36 cells or mouse and RT-PCR-sequencing for JE were conducted at the Division of Arboviruses in the Republic of Korea CDC. This laboratory developed a commercial-nested RT-PCR kit for JE virus detection.

For bacterial antigens, latex agglutination, PCR, and serotyping of the isolates were performed at the other division in the Republic of Korea CDC.

2.4.5.9 Viet Nam

_Ha Noi._ The National Institute of Hygiene and Epidemiology performs JE testing for the northern part of Viet Nam. This laboratory developed an in-house MAC ELISA kit that recently was evaluated in NIID, Tokyo. National data of viral encephalitis cases by region shows that more cases were detected in the northern part of Viet Nam during the 1990s. This
laboratory performed JE testing for 3738 samples during the period 2001-2008 and detected 1445 positive samples (38.6%). Among 284 samples collected from three sentinel sites during the period 2005-2006, 83 showed positive (29.2%). The age-wise analysis of JE cases shows that an age group of 5-9 years represents 34.95% followed by an age group of 10-14 years (31.34%). After introducing JE vaccination into the National Expanded Programme on Immunization (NEPI), the age distribution of JE cases has shifted towards older children. More male children are infected than female children. The JE vaccine coverage rate in Viet Nam during the period 2003-2007 ranged between 91.7% and 95.9% in targeted regions. However, this vaccination only covered between 18% and 52% of the target population in Viet Nam.

**Ho Chi Minh City.** The Arbovirus Laboratory at the Pasteur Institute, Ho Chi Minh City performed JE testing for the southern part of Viet Nam. This laboratory also worked out and used an in-house MAC ELISA JE kit for diagnosing JE along with HI, PRNT, virus isolation, and RT-PCR for CSF samples. The number of reported AES cases during the period 1998-2007 ranged from 420 to 694 annually. Laboratory confirmed cases by JE MAC ELISA were 1140 cases during the period 1999-2007. Six JE strains were isolated from sentinel pigs during the period 2005-2006 and four strains were from mosquitoes in 2005. By 2010, it was planned to vaccinate all target populations in all provinces and districts.

2.5 **Laboratory Integration and Other Issues**

2.5.1 Integrated laboratory services for vaccine preventable diseases (VPD) surveillance

Dr Olen Kew from the United States of America CDC presented key points for integrated laboratory surveillance for vaccine-preventable disease. Global VPD surveillance has been built on a polio model with solid infrastructure and with many lessons learned so far. There was concerted pressure to expand laboratory capacity rapidly in VPD, but current capacity for expansion is limited. Building a laboratory network is a complex and long-term process.

Global Framework on Immunization Monitoring and Surveillance (GFIMS) has been developed by WHO in collaboration with its global partners and endorsed by the WHO Strategic Advisory Group of Experts (SAGE) for Immunization. In the Guiding Principle 4, linkage with other VPD, non-VPD surveillance, and monitoring systems and with the private sector is emphasized. Monitoring and surveillance strategies would be: (1) promote case-based surveillance; (2) build on and expand existing laboratory networks; (3) build capacity at the country level for VPD surveillance, programmatic monitoring, and for monitoring vaccine safety; (4) promote the development of a common platform of VPD and other infectious diseases monitoring and surveillance functions; (5) promote the collection, analysis, interpretation, and use of programmatic information; and (6) coordinate with other disease prevention and control activities to ensure appropriate and timely use of vaccines.

There also were challenges to VPD surveillance networks: (1) trying to do too much too fast; (2) underestimating the complexities of network building; (3) global resources were still far too limited; (4) new networks must come on line while original networks still maintain their functions (polio eradication was not yet achieved); (5) competition for a limited pool of well-trained staff; (6) inadequate attention to VPDs in some regions; and (7) insufficient support to achieve clearly stated WHO goals.

When setting up a VPD surveillance system, the following must be taken into consideration: (1) a disease control objective; (2) a surveillance objective (disease burden); (3) properties of the disease agent, e.g., clinical presentation/attack rates, antigenic
types/variability, specimen transport requirement; and (4) local context/infrastructure and resource needs.

In addition to polio and measles/rubella, JE/AES, rotavirus, influenza, pneumonia, and meningitis surveillance networks were established or were being formulated. However, not all VPD surveillance can be integrated, and one size does not fit all. There are areas for integration to promote efficiency and synergy, but not all VPDs are included in the EPI programme. Objectives of specific disease surveillance can define system structure. Many laboratories have experience in working with other disease programmes aside from the traditional EPI diseases. Expansion to other diseases would be possible but will require resources, including more LabNet coordinators and strong links to disease control programmes; they are critical.

2.5.2 Shipping requirements of infectious materials

Dr Bruce Thorley from the VIDRL, Melbourne, gave a presentation on the safe transport of infectious substances. He discussed the safe transport of specimens and isolates, the international transport of infectious substances and risk groups of pathogens and infectious substances. Carrying infectious substances aboard aircraft as check-in or hand luggage is strictly prohibited by law.

Category A infectious substances are those that can cause permanent disability or life threatening or fatal diseases to humans or animals when they are exposed to them. These substances should be shipped as UN 2814 infectious substances affecting humans. Category B Infectious substances are those that do not meet the criteria for category A, and they can be shipped as UN 3373 Biological substances, category B. One example is stool specimens to be tested for poliovirus isolation.

Pathogens with minimal hazard, inactivated or neutralized pathogens, DBS, or environmental samples considered not to pose a significant risk (food or soil) are exempt specimens. They may be shipped using category B packaging with the label, "Exempt Human Specimen" on the exterior.

For category A infectious substances, the maximum volume for shipment is 50ml. Triple packaging with primary receptacle (tubes), secondary receptacle (plastic container), and rigid outer packaging (cardboard box) should be prepared for all infectious substances for international transport. Documentation such as customs declarations or commercial invoices, dangerous good declarations, import permits, and airway bills should be prepared. Category A substances should follow PI 602 and category B substances should follow PI 650 packing instructions. Tubes with external threads with more secure seals for shipment and storage of infectious substances and biological substances are recommended.

2.5.3 Report of joint WHO-United States of America CDC International Conference on Health Laboratory Quality Systems

Dr Gayatri Ghadiok from the regional office briefed at the conference, which was held on 9-11 April 2008. A total of 184 participants from 69 countries attended. Five laboratory experts from the Western Pacific Region from the Philippines, Viet Nam, Cambodia, and Fiji attended the meeting. Experts from Malaysia, New Zealand, and Australia made presentations. Laboratory quality is important for making therapeutic decisions and for disease prevention programmes. It also has financial implications. Laboratories play a vital role in every aspect of health service delivery and need strong support. There are challenges in implementing quality systems in developing countries.
As a guideline: (1) governments should organize infrastructure to support a national laboratory of quality standards; (2) the national laboratory should establish quality standards; (3) laboratories should implement quality improvement system programmes; and (4) underlying problems should be addressed during pre- and post-analytical phases. Governments should: (1) establish the focal point in the Ministry of Health, and scientific and technical advisory system with professional bodies/organizations; (2) establish a national laboratory quality office with a responsible officer and authority; and (3) introduce measures to improve the quality of health laboratories, e.g. link the accreditation of the laboratory with the reimbursement of laboratory costs.

The introduction of the International Organization for Standardization (ISO) should be considered: ISO 15190 for medical laboratories and ISO/IES 17025 for all types of testing and calibrating laboratories.

Each country should establish its own set of standards based on ISO 15189. National laboratory standards should be taken into account and should include national regulations, organization of the laboratory system in the country, and resource constraints. Countries with limited resources can consider a staged approach.

To implement a laboratory QI system at the national level, national quality standards should be established, SOPs should be introduced, laboratory facilities and infrastructure should be provided, appropriate technologies, including IT systems, should be introduced, laboratories should be structured in a well-organized system with clear management responsibilities, and safety issues should be addressed properly with preventive and protective waste disposal and clean and tidy facilities. In addition, regular service and calibration and safety procedures for equipment should be established, training opportunities and education for laboratory staff should be available, long-term plans for the adequate supply of trained personnel should be formulated, QS and standards should be applied in every aspect of laboratory operation -- including the procurement of supplies and equipment - national and regional plans for quality standards (QS), internal quality control (IQC) and external quality assurance (EQA) introduction should be worked out, and a mechanism for monitoring also should be also created.

WHO’s role in laboratory strengthening would be: (1) promoting advocacy and information exchange; (2) producing strategies, guidelines, and technical documents; (3) providing training and capacity building; and (4) developing and formulating assessment tools. There are some areas with different programmes within WHO that can work together, including prequalification of quality reagents, biosafety and biosecurity in laboratories, and resource mobilization.

2.5.4 Future plans for the Western Pacific Region VPD laboratory network

EPI in the Western Pacific Region has well-established laboratory networks for polio and measles/rubella and is strengthening the JE LabNet in the Region. At the global level, a human papilloma virus LabNet has been established and laboratory networking for new VPD pathogens, including rotavirus and pathogens causing bacterial meningitis, also were planned. The existing VPD LabNet has standardized procedures for sample collection and transport, testing using a validated assay, a WHO manual, quality assurance (referral and confirmatory testing, proficiency testing, performance indicators, accreditation), and reporting systems. There are areas for collaborative activities to improve laboratory quality such as quality assurance programmes, biosafety, capacity building, standardization of equipment and reagents, shipping and laboratory data management, and capacity building.
The VPD laboratory network has a critical role to play in the VPD control programme. Laboratories are critical to VPD surveillance and early outbreak detection. Key components for a successful network include standardization, quality assurance, and collaboration, communication and coordination among LabNets, national surveillance programmes, and WHO.

Building up laboratory diagnostic capacities for bacterial agents: pertussis and diphtheria are required at the regional level and the designation of regional reference laboratories for pertussis, diphtheria, and tetanus were to be considered.

2.6 Report of Laboratory Side Meeting, 10 July 2008

The side meeting was held from 12:00 to 13:00, with the following attendees:

- Polio and measles GSL/RRL: Dr Hiroyuki Shimizu, NIID, Tokyo (polio); Dr Bruce Thorley, VIDRL, Melbourne (polio); Dr Xu (Zhang), China CDC, Beijing (polio); Dr Tashiro, NIID, Tokyo (measles); Dr Leydon, VIDRL, Melbourne (measles); Dr Wilina Lim, Center for Health Protection, Hong Kong (China) (polio/measles)
- Dr Paul Rota the United States of America CDC, Atlanta (measles)
- WHO staff: Esther de Gourville and Dr Fem Paladin, WHO HQ (polio), David Featherstone, WHO HQ (measles), and Youngmee Jee, WHO Western Pacific Regional Office (polio/measles).

The discussion was on:

2.6.1 Measles

Plans for 2009 measles laboratory training were discussed, as follows:

Cell culture and RT-PCR training (measles and rubella) can be conducted in Hong Kong (China) during the period April-June 2009 for Vietnam, the Philippines, Malaysia, and Mongolia.

ELISA, DBS training can be conducted in Cambodia for Papua New Guinea, the Lao People's Democratic Republic, Cambodia, the Pacific island countries and areas (Fiji). WHO HQ can also provide support for a facilitator.

China was planning its own training during September or October for polio and measles. Previous trainings were held as follows:

- China: annual national workshop;
- ELISA and DBS workshop in the VIDRL, Melbourne, May 2005 (with participation from the Lao People's Democratic Republic, Papua New Guinea, Cambodia, New Caledonia, Guam, Fiji, Viet Nam, and Mongolia);
- Onsite training, Cambodia, August 2005; and
- ELISA and cell culture, Government Virus Unit, Hong Kong (China), 2006.

Onsite training for Papua New Guinea and the Lao People's Democratic Republic was planned. Dr Michaela Riddell (VIDRL, Melbourne) was to visit Papua New Guinea in October for DBS follow-up training. Dr Chan Kwai Peng (Singapore) was to visit.
the Lao People's Democratic Republic in September for ELISA quality control (QC) and data management.

The designation of countries for confirmatory testing referral to Hong Kong (China) and Australia was discussed. Japan also was to receive samples from Mongolia. Since a referral pathway in the Pacific island countries and areas was not effective, the establishment of a referral pathway among them should be discussed again. It was recommended to use the 2009 PIPS workshop as an opportunity to discuss this issue and to emphasize the importance of communications and reporting.

For providing Vero/SLAM cells to network laboratories, NIID, Tokyo, has agreed that Hong Kong (China) and Australia also may ship the cells to national measles laboratories in the Region.

2.6.2 Polio

Polio real time ITD PCR training was planned for the period July–August 2009 in the VIDRL, Melbourne, once the method is finalized.

There was a request for standardization of (ITD) by sequencing. SOPs and a proficiency test panel also should be prepared at the global level.

The problem of high shipping costs from national laboratories to RRLs or the GSL was discussed. The possibility of using the polio surveillance budget was proposed.

For China accreditation in 2008, the plan was for seven provinces to be visited in October 2008. Dr Steve Oberste and Dr Paul Rota from the CDC will join the accreditation. JICA postponed the accreditation plan. The JICA team was to visit six provinces in October or November 2008. It was proposed that WHO should closely monitor and discuss the recruitment of the JICA accreditation team. NIID polio experts were to be selected as reviewers for the 2008 visit.

For the annual JICA training in 2009, NIID was to coordinate with JICA and the WPRO for selecting participating countries. Dr Bruce Thorley will be invited as a facilitator.

Dr Bruce Thorley presented ITD results of poliovirus tests from Malaysia and the Philippines.

3. CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

A regular laboratory meeting, preferably annual, will be held to meet the regional need for updating the requirements of the network laboratories and to share information in the Region.

3.1.1 Polio Laboratory Network

(1) New accreditation checklists for the National and ITD Laboratories have been distributed to the network laboratories and will be used for the accreditation review of all network laboratories, including the 31 provincial laboratories in China.
The timeliness of reporting ITD results from ITD laboratories to the regional office was emphasized. All ITD laboratories should report their ITD results within seven days (reduced from 14 days).

(2) Although all laboratories were encouraged to implement the new algorithm for virus isolation, each laboratory should decide whether to introduce the new algorithm. After the introduction of the new algorithm, laboratories can continue to perform polio neutralization tests on the isolates in conjunction with ITD testing by the RRL. With full support from WHO HQ, the WPRO will organize real time PCR training for ITD laboratories in the Region to formulate real time PCR methods in the Region.

(3) The timeliness of reporting cell sensitivity testing was emphasized. Ideally, all network laboratories should report results within 48 hours of the completion of testing to rectify possible problems as soon as possible.

3.1.2 Measles Laboratory Network

(1) Since the measles and rubella laboratory network was established in 2004, the laboratories played a critical role in achieving the regional goal of measles elimination by 2012. A new case-based laboratory reporting scheme, which would be used in all network laboratories, was distributed. However, there will be further improvements in this reporting form as feedback from network laboratories are received.

(2) The importance of obtaining measles genotyping data was emphasized since we were aiming for measles elimination by 2012. The laboratories were encouraged to share with WHO the genotype information about circulating measles and rubella strains by submitting it to the WHO genotype database.

(3) The importance of confirmatory testing was emphasized to validate the results of the NMLs. Confirmatory testing will be performed regularly for samples from all national laboratories in the Region to ensure the accuracy and quality of testing. The regional laboratory coordinator can be consulted about frequency and the number of samples for confirmatory testing.

3.1.3 JE laboratory network development and laboratory integration and other issues

(1) To provide the necessary technical support for JE control, a new laboratory network for Japanese encephalitis was to be established in the WPRO during 2008. Designation of GSL (1), RRL (1-2) and national laboratories was to be completed by the end of 2008. The first hands-on training was to be held to establish the laboratory network. The validation of JE kits used in the region was to be performed in 2009. Proficiency test panels for JE also were to be distributed in 2009 to ensure the proper performance of the JE laboratory network in the Region.

(2) There is an increased need for establishing laboratory networks for other vaccine-preventable diseases. Existing VPD laboratory networks for polio and measles/rubella should be considered for use as a new laboratory network and for a JE laboratory network in the Region. Designation of regional reference laboratories for confirming bacterial VPD in the region was to be considered.

3.2 Recommendations

Increasing the numbers of specimens and activities for the polio and measles laboratory networks and the introduction of new vaccines in the Region greatly has increased the workload for regional laboratory coordination. To meet these rapidly growing demands, two
additional regional laboratory network coordinators are needed: one with expertise in virology, the other with expertise in bacteriology. Specific recommendations were:

3.2.1 Polio Laboratory Network

(1) The new accreditation checklists for the NPLs and RRLs should be implemented among network laboratories, including the 31 provincial laboratories in China, as soon as possible. SOPs should be also revised to meet new requirements in the checklists. Emphasis on laboratory management and biosafety should be addressed accordingly.

(2) The use of the new test algorithm for virus isolation should be encouraged and the serotyping of poliovirus isolates should be continued. Laboratories using the new test algorithm should revise their SOPs and laboratory database and changes should be shared with the regional laboratory coordinator immediately. The management of a polio laboratory database can be challenging if both the traditional and new algorithms are used in the Region. Laboratory indicators that also can reflect the new algorithm should be added to the polio laboratory database for those laboratories that implemented the new algorithm.

(3) The introduction of ITD function should be considered in laboratories that frequently refer poliovirus isolates to RRLs to reduce the cost of shipment.

(4) In countries with a low number of AFP samples and low nonpolio enterovirus (NPEV) rates, supplementary enterovirus or environmental surveillance can provide additional information on the sensitivity of laboratory surveillance.

(5) Laboratories in Australia, Japan, China, Hong Kong (China), New Zealand, and Singapore performing ITD should consider establishing their capacity to perform the new real time PCR assays for ITD and VDPV screening, which was being worked out by the CDC.

(6) Regional training or a workshop on real time PCR should be conducted in 2009 for the six ITD laboratories and the Malaysian NPL.

(7) All NPLs without an ITD function, including the provincial laboratories in China, should forward positive samples to the RRLs as soon as possible. The same EPID number should be used at all times.

(8) Cell sensitivity testing should be performed regularly at least once midway through 15 passages and results should be reported to the regional laboratory coordinator within 48 hours of the completion of testing.

(9) Considering that the network had not yet introduced the new algorithm, the proficiency test for traditional virus isolation should be provided in 2008. The performance of any laboratory shifting to the new test algorithm should be evaluated with the new proficiency test (PT) panel and timeliness of reporting results.

(10) The conventional CDC PCR ITD assay should be used among ITD network laboratories until the real time PCR assays for ITD and VDPV screening are fully implemented and performance has been evaluated successfully.
(11) Because of the shortage of ELISA ITD reagents in the Region, VP1 nucleotide sequencing can be used as second method for ITD testing. A standardized protocol and proficiency test for this method should be worked out.

(12) Laboratories should maintain regular communications with national EPI or surveillance units and report laboratory data regularly, at least monthly, to the regional laboratory coordinator.

3.2.2 Measles Laboratory Network

(1) The new case-based laboratory reporting scheme should be used in all network laboratories where possible and network laboratories are requested to submit their monthly data for the previous month by the tenth. This reporting will include a line list and summary data for the year to date.

(2) As recommended by the Global Measles Laboratory Network Meeting (September 2007), new checklists for the national and regional reference laboratories that emphasize the timeliness and completeness of genotyping and sequencing of measles viruses should be used for the accreditation of network laboratories.

(3) Laboratories with the ability to perform isolation and molecular detection of measles and rubella viruses are encouraged to do so. Genotype information on circulating measles and rubella virus strains in all countries should be collected as much as possible and all countries are encouraged to collect genotypic information on measles and rubella strains by 2009. This data should be submitted to the WHO genotype database and also shared with the regional laboratory coordinator. Laboratories also strongly are encouraged to submit their sequence information to GenBank.

(4) As implemented in the new checklist, the results of virus detection and genotyping, if performed, should be completed within two months of the receipt of the specimens. The data for at least 80% of the samples appropriate for genetic analysis should be reported monthly to WHO.

(5) To ensure acceptable laboratory performances of the regional measles network, all network laboratories were to be reviewed for accreditation by 2009.

(6) A confirmatory testing mechanism should be established routinely for all national laboratories in the Region to ensure the accuracy and quality of testing. The number of samples or selection of samples to be referred to the RRL can be coordinated with the regional laboratory coordinator before samples are sent to the RRL. The results of confirmatory testing should be shared with the global and regional laboratory coordinators. Possible reasons for discrepancies in the results between the NMLs and the RRLs should be sought and immediate corrective actions should be taken in the NMLs.

(7) All network laboratories are encouraged to perform anti-rubella IgM on all measles IgM negative samples from acute fever and rash cases, as recommended by WHO. Laboratories should be prepared to receive and test specimens from congenital rubella syndrome (CRS) surveillance.

(8) National laboratories should keep all measles and rubella-positive samples for the global PT and for future virus identification. The regional laboratory coordinator should be contacted before disposing of any positive samples. Those laboratories with
positive serum samples with volumes of greater than 0.5 ml are encouraged to submit them to the WHO proficiency testing panel in consultation with the regional laboratory coordinator.

(9) Laboratory training or workshops focusing on measles and rubella virus isolation and molecular detection will be provided to strengthen the capacity of the laboratories in 2009.

(10) WHO will advocate for resources to strengthen the measles and rubella regional LabNet, especially for supporting the NMLs in priority countries.

(11) Non-validated measles and rubella IgM ELISA kits used in network laboratories, including subnational laboratories, should be evaluated using a validated panel of serum samples.

(12) A mechanism for sample referral among the Pacific island countries should be reviewed and re-established in the Region.

(13) Because IgM detection in serum samples remains the gold standard for the laboratory confirmation of measles, ELISA using DBS and oral fluid samples can be applied among countries with moderate to high measles incidence that have difficulty transporting samples to the nominated testing laboratory. Those countries first should consult WHO.

(14) Efficient reporting and communication systems should be established between national surveillance and laboratory staff. Network laboratories are encouraged to work with their surveillance colleagues to collect and test measles and rubella samples from all regions of the country.

3.2.3 JE laboratory network development and laboratory integration and other issues

(1) Potential GSL (1), RRL (1-2) and national laboratories should be identified by 2008. Terms of reference and governmental support will need to be negotiated.

(2) The final version of the WHO manual for the laboratory diagnosis of JE was to be distributed to the network laboratories when finalized. This manual should be used as a guideline among JE laboratories in the Region.

(3) Evaluation data on in-house and commercial JE IgM ELISA kits used in the region should be collected and validated by early 2009.

(4) A training workshop for the laboratory diagnosis of JE should be organized in the Region by early 2009.

(5) Proficiency test panels for JE should be arranged for the network laboratories in the Region by 2009.

(6) Laboratory capacity to support acute encephalitis syndrome surveillance and to detect bacterial antigens and JE should be established in the Region. The laboratory capacity for confirming bacterial VPDs will be strengthened by designating regional reference laboratories to confirm bacterial VPD in the Region.
(7) Confirmatory testing mechanism similar to measles and rubella should be established to ensure the accuracy and quality of testing.

(8) A formal accreditation system to evaluate the laboratory performances of the network laboratories should be established in collaboration with HQ and WHO Regional Office for South-East Asia.

(9) Pre-existing VPD laboratory networks such as polio, measles and rubella should be used to establish the new laboratory network as much as possible for JE diagnosis in the Region. Validated ELISA kits will be provided to priority countries.

(10) A Laboratory reporting system for JE network laboratories will be developed and distributed by 2008. A laboratory information system to facilitate data management should be established by early 2009.
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**SEVENTEENTH MEETING OF THE TECHNICAL ADVISORY GROUP ON IMMUNIZATION AND VACCINE PREVENTABLE DISEASES IN THE WESTERN PACIFIC REGION:**

**Laboratory Network Sessions**

Manila, 7-9 July 2008
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