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 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 re-established 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 those 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.
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.
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.

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.

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

### 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.