

The Fourth WHO Global Measles and Rubella Laboratory Network Meeting

28 to 30 August 2006, WHO, Geneva

Summary and Recommendations

Global and Regional Summary

The measles and rubella laboratory network consists of almost 700 laboratories serving 164 countries. Most Network laboratories are integrated into their national public health surveillance programmes and are often testing for multiple other diseases, including HIV, Hep B, polio, YF, JE, seasonal influenza as well as bacterial and parasitic diseases. Measles and rubella testing is sometimes just a small component of a Laboratory's overall activities, particularly in countries with good measles and rubella control. The LabNet expansion phase has slowed recently but new laboratories have been established in countries which have recently strengthened surveillance for measles. Over the past 12 months laboratories have been set up and laboratory staff trained in; Nigeria (x6 laboratories), southern India, Libya, Somalia, Afghanistan, Djibouti, Mozambique, Gabon and Madagascar.

The strength of the measles and rubella LabNet is in the establishment of standardized testing and reporting procedures. Most laboratories are using well validated IgM assays and are following standardized validation procedures and staff having participated in WHO coordinated training. Globally, more than 120,000 serum samples were tested for measles IgM in 2005 and more than 73,000 in the first 6 months of 2006. Most laboratories are reporting at least 80% of these results within 7 days to their national programmes.

An annual proficiency testing programme has been in place since 2001. Of the 143 laboratories which participated in the most recent test, 98% achieved the pass rate of 90% or better. In 2006-07, laboratories in all 6 WHO regions will participate in the same proficiency testing programme for the first time. A comprehensive revision of the 1999 Measles laboratory manual is in the final stages of publication. The manual has an increased emphasis on rubella diagnosis, cell culture, virus isolation, quality assurance, and the 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 CDC for financial support. Technical support is provided by a small number of Global Specialized and Regional Reference laboratories and coordination is provided through coordinators in each of the regions and in HQ. Almost all Laboratory

coordinators have the added responsibility for other surveillance VPD LabNets, including; YF, JE, HPV and/or Rotavirus.

The region of the Americas' laboratories are facing the challenge of confirming measles and rubella cases in countries which have very low incidence or have eliminated indigenous circulation of these diseases. As the PPV for IgM assays is low under these circumstances, countries are faced with the challenge of how to resolve false positive results and vaccine related IgM responses. All national laboratories in the region now use IgG assays to help resolve difficult to diagnose cases, and rubella avidity testing is available in all regional reference laboratories and also 3 national laboratories. PCR and virus isolation techniques have been established in all the RRLs, 9 national laboratories and some SNLs.

The African measles LabNet comprises 36 national and 3 regional laboratories and is fully integrated with yellow fever testing where appropriate. Most laboratories are functioning at a high level of proficiency and meet timeliness of reporting criteria. A systematic programme of validation of samples is operating in the region and laboratories are required to report IgM results and kit monitoring data to the regional office monthly. As a result of decentralization in the African region, laboratory coordinators will be based in each of the three sub-regional Inter Country Support Team (ICST) centres from mid-September 2006. The impact of this reorganization on the coordination of the network will need to be carefully monitored.

The Eastern Mediterranean region (EMR) has set a goal of measles elimination by 2010. The LabNet is in the consolidation phase with all countries having established a measles national laboratory except Djibouti which will do so in September 2006. Eleven of the 22 National laboratories have the capacity to perform measles and rubella virus isolation. Both RRLs in Oman and Tunisia have access to PCR technology. The Tunis laboratory performs measles sequencing for the region. Proficiency of most of the National laboratories is high, with 17/18 passing the latest proficiency test. Only 10 laboratories implemented the sample validation performance criterion in 2006 but all these achieved greater than 90% accuracy. Six countries passed the accreditation review and more countries are anticipated to go through accreditation process before the end of 2006. In December 2005, a training workshop was held in the Cairo National Measles Laboratory for ELISA and measles virus isolation techniques. Participants from eight national laboratories attended. Challenges for the EMR LabNet include; encouraging laboratories to integrate their data with their surveillance programmes and to increase the number of samples for validation and virus detection being sent to the Reference laboratories. It is anticipated that the establishment of another regional reference laboratory in EMR would facilitate the shipment of samples for retesting and strengthen the virus detection capabilities in the region.

The South East Asian region is focusing on sustainable measles mortality reduction though Bhutan, Maldives and Sri Lanka are implementing elimination strategies following successful mass campaigns. All countries have established measles national laboratories with India nominating 3 to cover southern states. Timor Leste's laboratory has yet to function fully. One RRL has been established in Bangkok and has the ability to perform virus isolation and molecular techniques. As measles surveillance activities increase, another RRL will likely need to be established in 2007. National surveillance programmes currently focus on investigating outbreaks and recommend collection of 5-10 blood samples from each outbreak for laboratory testing. Six laboratories have now initiated virus culture capabilities and this will be expanded in 2007. A laboratory based data management software has been developed and was distributed to laboratories following a laboratory training workshop in June 2006.

The European region (EUR) has established measles and rubella elimination targets for 2010. The LabNet is well established and consists of 70 laboratories, including 20 sub-national laboratories, three RRLs and one Global Specialized laboratory and tests more than 35,000 IgM samples per year. Most laboratories are meeting performance indicators and the accreditation programme has been strengthened over the past 2 years. No laboratory failed the proficiency test in 2006. Some of the challenges for the EUR LabNet in the future are; evaluating the impact of privatised decentralized and reorganized health care systems on measles surveillance, maintaining resources to support low GDP countries with assays and other consumables, and the distraction of avian influenza and other "new interest" diseases on routine laboratory activities.

The Western Pacific region (WPR) recently established a measles elimination target date of 2012. The WPR LabNet is now established and more than 35,000 serum samples were tested in 2005, 30,000 of which were in China. A measles outbreak in Fiji in April 2006 was confirmed by the recently designated measles laboratory in Suva and virus genotyped as H1 by the RRL in Australia. The measles surveillance programme in other Pacific Island Countries is not yet fully functional and most of the designated laboratories have yet to receive samples for testing. All WPR national laboratories passed the proficiency test in 2005, confirmatory testing has been implemented by 6 laboratories and a good start has been made with the accreditation process. Training workshops were held in the Hong Kong laboratory for ELISA and virus culture techniques in March 2006 and in the China CDC for China's 31 Provincial laboratories in October 2005. Challenges for future development of the WPR network include; improving sample collection for IgM and virus detection, validation of test kits used in sub-national LabNets and an improved timeliness and completeness of laboratory reporting.

All regions have made considerable progress with completing the accreditation process in their national laboratories. However, implementing the requirement for shipping sera to reference laboratories for validation and for transporting

viruses or clinical material to sequencing laboratories for genetic analysis has considerable operational cost implications for the network.

Due to the potentially heavy workload of the accreditation process, laboratory coordinators are encouraged to only perform the onsite review component for each laboratory every 3-4 years, but may need to visit laboratories which face challenges in meeting performance or quality indicators more frequently. Though the WHO laboratory coordinators can provide advice, maintaining the quality of the sub-national laboratory network is largely the responsibility of the National Laboratory in countries where geographical size, population or logistical challenges necessitate the establishment of sub-national laboratories.

Recommendations:

- 1. Additional funding is needed to maintain the strong quality indicators of the LabNet and to improve virus detection, particularly to facilitate sample collection and transportation to the National and Regional Reference Laboratories. In order to increase the funding base, Regional Offices should explore sources for funding, preferably from donors that can make a long-term funding commitment. Laboratory coordinators should take advantage of opportunities to build advocacy for LabNet and ensure that laboratory surveillance is included in Regional surveillance plans. **Action: All Laboratory Coordinators, Ongoing.***
- 2. The Measles and rubella laboratory manual is in the final stages of publication. Translation into the predominant languages used in the laboratory network is seen as a high priority and should be completed as soon as possible. **Action: WHO HQ Coordinator, ASAP***

Status of Alternative testing

Dried blood and oral fluid samples continue to be seen as attractive alternatives to the "gold standard" serum sample for measles IgM detection, under specific circumstances. The UK has used oral fluid (OF) sampling for measles mumps and rubella surveillance successfully for almost 10 years but commercially available assays for rubella IgM detection in OF samples have been released on the market only recently and have not been fully validated. Dried blood (DB) sampling using the Behring IgM assay has been shown to have good sensitivity and specificity (>99% and >96% respectively) for measles when compared with serum, in studies of more than 800 cases, but rubella data is still limited.

J Icenogle presented a Peruvian study where dried blood, oral fluid and serum samples were collected from 237 cases during a rubella outbreak and most samples were collected within the first 3 days after rash onset. CDC found 90% sensitivity and 93% specificity when comparing serum and dried blood samples. Oral fluid testing had yet to be completed. All dried blood samples were tested

after 30 minutes of extraction and 30 minutes centrifugation and tested under the standard serum protocol in a Behring IgM assay. EIA absorbances for rubella dried blood samples were approximately 83% of the paired serum sample, compared to about 97% for measles IgM under equivalent conditions found during the Uganda 2004 study. The explanation for this lower absorbance ratio is not fully understood.

A measles and rubella field study in Uganda was reported by J Bwogi, where dried blood, oral fluid and serum samples were collected from cases under routine surveillance conditions. Samples were shipped to reach the laboratory within 3 days, dried blood at ambient temperatures, and serum and oral fluid under reverse cold chain. Data were only available on the measles tested samples. Sensitivity and specificity results for dried blood vs. serum samples showed 83.9% and 98.4% respectively, and for oral fluid vs. serum, 91.5% and 91.8%. Three of eight oral fluid samples and none of nine dried blood samples were positive by a measles virus detection RT-PCR test.

Some of the challenges in implementing dried blood testing in AFRO were described by A Dosseh. Some difficulties were reported with receiving adequate volumes of dried blood and some laboratories were concerned about the increased processing time required to extract serum from dried blood samples. The Sierra Leone surveillance programme has moved entirely to dried blood collection as international couriers refused to transport serum samples due to a fear of Lassa fever virus. Sierra Leone's dried blood samples are tested in Senegal and 56% were measles IgM positive. All measles negative samples were also tested for rubella IgM, and 46% found positive.

Data were provided by M Riddell, where rubella IgM in dried blood samples from 88 rubella cases in Melbourne were tested using extraction and assay modifications published by Riddell et al, 2002. Results showed sensitivity of 92.9% to 96.7% compared with serum, depending on whether equivocal were considered negative or positive (specificity 100% for both).

A study by Chibo et al, 2005, showed OF samples dried on filter paper could be used for detection of measles RNA by RT-PCR methods after storage at temperatures up to 37°C for 7 days though there was ~30% loss of activity compared with freshly tested samples.

D Brown reported preliminary data from a rubella study in Vellore, India. Confirmation of rubella infection using detection of IgM in oral fluid from 54 suspected post natal rubella cases showed 95.5% sensitivity. For 111 suspected CRS cases sensitivity and specificity was 100% for both.

A comparative study of the absorbances of Whatman 3M and 903 and Schleicher and Schuell 903 filter papers used for dried blood collection was reported by M Riddell. Whatman has taken over the Schleicher and Schuell Company (S&S)

and now produces a Whatman 903 paper which appears similar to S&S 903 paper. Evidence was provided showing that Whatman 3M paper absorbs blood more slowly and has significantly lower OD levels than both S&S 903 and Whatman 903 when used for measles and rubella IgG detection. S&S 903 and Whatman 903 (Cat No. 10535097) had equivalent ODs for measles IgG and similar but not equivalent OD levels for rubella IgG.

Oral fluid stability data provided by D Brown, HPA, showed measles IgM was stable in Oracol tubes for up to 7 days at 37°C, though the number of samples tested were small. One of four individuals showed a 65% drop in OD over 7 days at low concentrations of IgM, though a positive result was still recorded. Spiked RNA in oral fluid samples was shown to be stable for a similar period of time and temperature. Oral fluid samples dried onto S&S 903 filter paper or onto the Oracol sponge was stable for 7 days at 37°C also, though IgM was not evaluated.

Evidence was provided by P Rota confirming that measles RNA could be amplified from IgM positive oral fluid samples up to 4 weeks after rash onset. In a small study of the stability of dried oral fluid samples 5 IgM positive OFs were dried in situ and incubated for 3 weeks at 4°C, 22°C and 37°C. RNA could be detected in 5/5, 3/5 and 1/5 samples, respectively. The RT-PCR protocol used for both studies was a standard, single step 40 cycle amplification, followed by followed by agarose gel electrophoresis and ethidium bromide staining.

The programmatic implications of alternative samples were presented by D Featherstone. The cost of collection and testing serum, dried blood and oral fluid samples are similar, however considerable cost savings are made if samples can be transported without the need for reverse cold chain and/or the sample is not required to be packaged under "diagnostic sample" conditions by IATA regulations. Dried blood is not classified as a "diagnostic sample" by IATA. Introduction of alternative samples for routine testing would require the development of specific quality assurance programmes, including proficiency testing.

CDC suggested that regions in the elimination phase, and which have an established fever and rash case-based surveillance system, would not benefit from universal introduction of dried blood or oral fluid sampling methods, but these techniques could be introduced on a case-by-case basis, especially where:

- Timeliness of specimen transportation from remote and/or difficult to access areas to the laboratory conducting the serological analysis could be improved or even achieved
- These could be used for the inexpensive transport of serum samples for validation testing

- Collection of oral fluid in addition to serum could improve efficiency of case identification and virological surveillance

However alternative samples may present problems (sensitivity, specificity and sample volume) if there is a need for differential diagnosis after measles and rubella are ruled out in rash and fever cases.

Recommendations: Alternative sample collection procedures:

3. *Though encouraging stability data for measles IgM and RNA in oral fluid and dried blood was shown, data for oral fluid is limited to several small studies. Data from two field trials investigating the feasibility of using dried blood and oral fluid for measles and rubella surveillance were presented, but testing for rubella IgM in oral fluid had yet to be completed. It is recommended that a small working group should be convened to develop a document demonstrating the practicalities of using alternative testing procedures for measles and rubella surveillance and provide guidelines for the circumstances under which they could be used. **Action: WHO HQ and RO, GSLs and key RRLs. By first quarter 2007.***
4. *Before this document can be produced the following data should be made available:*
 - a. *Results from the testing of oral fluid samples for rubella IgM in the Peru rubella study. **Action: CDC***
 - b. *Clarification of specimen collection, extraction and testing procedures (serological and molecular) from the 2006 Ugandan study, to ensure that results are comparable with other studies. **Action: UVRI, Uganda***
 - c. *Testing of Rubella IgM in measles negative sera collected during the 2006 Uganda study. **Action: UVRI, Uganda and CDC***
 - d. *Stability data for RNA and IgM in oral fluid and dried blood covering a range of temperatures to a maximum of at least 37-40°C, and for periods covering 0-2 weeks. **Action: CDC and HPA***
 - e. *Stability of IgM in serum dried onto filter paper and stored at ambient temperatures for 0-2 weeks for validation testing. **Action: CDC and HPA***

Data management

An update of the collection and reporting of global measles data was presented by M Gacic Dobo. Though progress was reported to be slow, 3 of 6 regions are now reporting data in an appropriate format, although reporting frequency is intermittent. The proposed global output includes: monthly aggregated data for field and laboratory results, global measles outbreak map, planned immunization (SIA) activities database, and genotype information. Access to this data will likely be web based. A global meeting will be held in September of data

managers and measles surveillance focal points to resolve outstanding issues concerning standardization and timeliness of reporting.

An example of the laboratory genotype database was presented by D Featherstone. The database now comprises more than 1200 measles viruses and the sequencing laboratories from 4 regions have contributed to it. The current data elements and database format was agreed upon although a more explicit description of the variables (date submitted, submitter laboratory, validation) was needed. Extensive discussions were held on: the accessibility of the database, timeliness of reporting and accessible the accessibility of non-public domain sequence data. It was suggested that some laboratories would be reluctant to contribute sequence information, or to the database at all, if public domain access occurred. It was stated that the more frequently the data was updated – the more useful the information was for programmatic application and G Tipples reported that Canadian public health physicians are now demanding real-time genotype data on global measles transmission.

Recommendations on Data Management:

5. *Sequencing results should be reported to the laboratory contributing the virus or sample as soon as completed by the sequencing laboratory with a copy to the regional laboratory coordinator. **Action: All sequencing laboratories, ongoing***
6. *Each sporadic case sequenced and representative strains from outbreaks should be included in the WHO genotype database (in the agreed upon standard format to facilitate automatic updating) and the year to date database sent to the regional office monthly, including zero reporting, by the 7th of each month or according to regional timelines for other laboratory data reporting. **Action: All sequencing laboratories, ongoing***
7. *The Regional Office will send an aggregated line-list, year to date, genotype database compiled from each of the sequencing laboratories in the region to WHO Geneva monthly, by 15th of each month. **Action: All Regional Coordinators, ongoing***
8. *WHO Geneva will develop two updated global sequence databases to reflect the needs of the measles control programme (genotype information) and the WHO LabNet (sequence information). These databases will be posted by the 21st of each month to :*
 - a. *A public access WHO HQ IVB website with genotype and epidemiological data in an electronically searchable format. GenBank accession numbers will be listed, if provided by the sequencing laboratories, but sequence data will not be available.*

b. *A password restricted website, with electronic search capacity. Non-public domain sequence information will be accessible, if provided by sequencing laboratories. **Action: WHO HQ, ongoing***

9. *It is strongly recommended that sequence data be submitted to GenBank and the accession number submitted to the public domain database, but will not be considered obligatory. Sequence information that has not been submitted to GenBank is also strongly recommended to be submitted but this information will be made available only in the restricted access database. **Action: All sequencing laboratories, ongoing***

10. *Any use of sequence data by a second party is expected to follow the protocol of consulting the contributing and/or sequencing laboratory before any publication occurs. A written protocol based on one HPA formulated for the ELSM project will be developed and shared with all laboratories requiring access to the database. **Action: WHO HQ, HPA, All laboratories, Ongoing***

Molecular epidemiology

Sequencing laboratories in each of the WHO regions reported recent measles and rubella molecular epidemiological information. As an indication of the increased activity in this field more viruses have been reported to the WHO genotype database in the first half of 2006 than for all of 2005. Many of the gaps in measles genotype information have been filled in the past 2 years due to the improvement in surveillance of measles viruses globally and the increased capability of laboratories in the LabNet. Efforts to improve virus surveillance include: virus detection training workshops, widespread distribution of Vero/SLAM cells, introduction of simple to use throat swab devices, and the use of oral fluid and serum for virus sequencing purposes.

In the past year measles sequence information, in combination with thorough epidemiological investigation, has allowed the tracking of virus transmission pathways across countries and regions, and provided information on whether outbreaks were likely to have been due to imported or indigenous viruses. Information on more than 175 measles viruses has been submitted to the WHO genotype database during the first half of 2006 and represents 10 different genotypes identified from more than 20 countries.

Of special note is: The transmission of one strain of B3 measles virus in 2006 which was tracked from Nigeria to Kenya to the USA, Canada, Mexico and several countries in Europe. A different B3 strain was found in small outbreaks in several European countries and subsequently in Australia and Venezuela. A large D6 outbreak (>40,000 cases) in Ukraine from late 2005 and 2006 was subsequently detected in most of the neighbouring countries and wider Europe.

A large D4 measles outbreak in Romania (>7,000 cases) spread throughout the country over 2 years and then onto several neighbouring countries.

Several laboratories have reported detection of a small number of genotype A measles viruses in the past few years and discussion took place as to their likely source, vaccine or wild type. Use of vaccine strain (virus or RNA) as a positive control for RT-PCR was highlighted as a potential source of contamination and confusion. G Tipples mentioned that his laboratory uses genotype E virus as their positive control to address this issue.

Rubella molecular surveillance has improved since the rubella nomenclature meeting in 2004, but many gaps still exist. The LabNet has standardized on the use of Vero/SLAM cells as a safe, sensitive cell line for isolating both measles and rubella viruses. Virus detection protocols using fluorescent and non-fluorescent procedures have been developed by CDC and are outlined in the new laboratory manual. Through the limited global rubella surveillance, a pattern appears to have emerged:

- Clade 2 viruses have not been found circulating in the Americas,
- Some genotypes are geographically restricted (e.g. 1C, 1D)
- Some genotypes are widely distributed (e.g. 1E, 1g)
- Genotype 1E was first observed in 1997 and is now widely distributed
- Africa, Russian Federation, the Middle East, Southeast Asia and the Americas have gaps in molecular surveillance
- Japan has detected a virus strain circulating between 2001 and 2004 which appears to be unrelated to other known genotypes
- Provisional genotype 1g appears to fall into 4 sub-groups when using the recommended 739nt sequence window

CDC reported that a data management/bioinformatics position will be created shortly to provide molecular surveillance outputs and coordinate database management in collaboration with other sequencing laboratories, WHO HQ and regional Offices.

Side Meeting: Rubella Genotype Characterization

A side meeting examining recent developments in the genetic analysis of rubella virus was held for interested participants. The following conclusions were agreed on.

- Guidelines for rubella nomenclature should be modified to remove the requirement that the phylogenetic tree of the individual structural protein coding sequences for candidate reference viruses for a new genotype have the same branching pattern as the full structural protein coding region. (Individual coding sequence analysis should still be done for candidate reference viruses to look for variant phylogenetic behaviour of

individual candidate reference viruses compared to other candidate reference viruses.)

- The recognized and provisional genotypes will not be changed partly because of concerns about the validity of the current methods of assigning genotypes. Data supporting elevation of provisional genotype 1g to a full genotype 1G and data supporting recognition of a new genotype from Japan were presented.
- Specific issues regarding the current recommended methods for identification of rubella genotypes were raised which will be addressed by rigorous analysis of current recommended methods by GSLs and interested RRLs. Recommendations for changes in nomenclature which may result from this analysis will be considered in the next year.

Recommendations: Measles and rubella molecular epidemiology

- 12. Regional laboratory coordinators will coordinate the production of a brief regional report on the distribution of measles and rubella genotypes in their regions. The report will be coordinated through CDC and WHO HQ and should be ready for publication in the WHO WER in the final quarter of 2006. A reporting template will be supplied by CDC. **Action: All Laboratory coordinators, sequencing laboratories, GSLs, Draft by October 2006.***
- 13. To encourage programmatic knowledge of measles and rubella sequence data, all sequencing laboratories are strongly recommended to use the standard WHO nomenclature for all viruses submitted to the genotype and sequence databases, when labelling viruses on dendrograms, and in all publications. **Action: All sequencing laboratories. Ongoing.***
- 14. A proposal to describe strain variation within measles genotypes that have extensive genetic diversity will be developed. Designation of additional reference strains for these genotypes will be circulated to the RRLs, GSLs and WHO Strain Banks and the recommended reference strains will be identified in the WER and on the WHO web site. **Action: GSLs, sequencing laboratories, Draft by Dec 2006.***
- 15. GSLs and other rubella sequencing laboratories are encouraged to generate more structural protein (SP) sequences and share these with other interested laboratories in the network. Results of analysis of these sequences will be shared in a timely manner with the LabNet. **Action: All sequencing laboratories. Ongoing***
- 16. GSLs and interested RRLs will consider the issues raised regarding current methods for identification of rubella genotypes. Any*

*recommendations for modifications resulting from this analysis will be agreed upon by a subgroup of GSLs and other rubella sequencing laboratories. **Action: All sequencing laboratories. First quarter 2007***

17. *Efforts should be made to collect as much epidemiologic and laboratory data as possible from cases of genotype A measles viruses to determine whether their origins are related to vaccine strains or to indigenous circulation. (These should be reported in the monthly update to WHO). **Action: GSLs and all sequencing laboratories. Second quarter 2007***

CRS and Maternal Rubella detection

Confirming CRS cases

D Brown presented some of the challenges in confirming CRS cases based on experiences in the UK and from a large CRS study in Aravind, India and literature reports. It is accepted that IgM in CRS cases diminishes with time after birth. Generally, from birth to 3 months of age 100% of cases have detectible IgM; 3-6 months of age ~75% and 6-12 months 50%.

A combination of tests can assist with the final diagnosis of CRS and these can include; IgM, IgG of mother and/or baby, IgG avidity index, and virus detection. It was stated that care should be taken in interpreting high IgG avidity in under 3 month old suspected cases as this result could be confounded by maternal antibody.

In the Aravind study, 4 commercial IgM assays were compared and Behring showed the highest sensitivity and specificity (100% and 99.82%), where the "gold standard" was the consensus of at least 3 out of 4 assays (equivocals considered negative). A good correlation was found between rubella RNA in eye lens material and IgM and/or IgG positivity in 0-11 month old suspected cases.

Maternal rubella detection

L Grangeot-Keros presented her experiences at the Antoine Bécclère Hospital, Paris, in confirming maternal rubella infection. She reported that no one assay can provide conclusive results for all cases though using a combination of serological tests and virus detection can contribute to the final diagnosis. Some experiences reported included:

- IgM is always detected after a primary infection and can be detected from day of rash to 1-2 months after rash onset.
- After vaccination IgM may persist for up to 6 months or even years. Measuring the extinction levels of IgM can be helpful in differentiating recent infection from past vaccination. Stable levels of IgM 3-4 weeks apart practically exclude a primary infection.

- IgG develops quickly and usually persists for lifetime. Seroconversion is not always an indication of recent infection and stable levels of IgG are not always an indication of past infection. IgM testing in both cases can assist with the final diagnosis.
- IgG and IgM can both be elevated through non-specific stimulation of the immune system, and IgG can undergo a significant rise through reinfection.
- IgG avidity testing can also contribute to the final diagnosis and maturation of avidity increases at different rates after primary infection or vaccination. In one study avidity rarely exceeded 85% 6 months after vaccination whereas maturation rarely fell below 85% 6 months after primary infection.

The status of CRS surveillance in the Region of the Americas was reported by J Icenogle following his participation in a PAHO meeting in July 2006. The meeting concluded that CRS surveillance will contribute to the documentation of the interruption of endemic transmission in countries and in the Region. It was recognized that laboratory confirmation of suspected CRS cases is critical and that the challenge for the LabNet will be to develop a practical guide for specimen collection, use and interpretation of laboratory test results and work in collaboration with surveillance colleagues.

Recommendations: CRS and Maternal Rubella

*18. A small working group will develop a testing algorithm for guiding investigations into diagnosis of CRS and maternal rubella to allow regions/countries to make decisions on how to handle these challenging diagnostic situations. **Action WHO HQ, GSLs. By Dec 06.***

Laboratory Quality assurance Status

J Leydon, VIDRL, Australia reported on the proficiency testing programme for 2005-06. All regions participated except for PAHO which participates in a proficiency programme developed and coordinated through CDC. Laboratories which participated in the VIDRL 20 sample panel mostly used Behring assays and 98% of the 143 laboratories achieved a score of 90% or more. During 2006-07, PAHO laboratories will participate in the VIDRL proficiency panel and the global panel will include a more representative number of rubella IgM samples. A review of the proficiency testing programme by VIDRL showed that 44% of laboratories indicated that the volume of each panel sample was too small for testing both measles and rubella and 30% of laboratories stated they did not receive global feedback from their coordinators. An evaluation of the stability of the PT panels showed that storage at 4°C over 12 months had minimal impact on the OD values of individual sera and no definitive results changed.

The PAHO proficiency programme was described by J Rota, CDC. The most recent panels consisted of 5 for measles and 5 for rubella and almost all of the 21 laboratories participating used Behring IgM assays for both measles and

rubella. One laboratory achieved 90% in both measles and rubella because of the failure to run appropriate controls. All other laboratories achieved 100% for both measles and rubella. One of the challenges in providing the PAHO PT is in shipping the panels to individual countries. In 2006-07, when PAHO uses the global panels, CDC will continue to ship the panels to countries in the Americas.

The progress made with implementing quality assurance in the PAHO LabNet was reported by M Siqueira and AM Bispo. Accreditation reviews started in 2006 and though the validation criterion was not evaluated it will be implemented in 2007. Some of the challenges laboratory found in establishing in-house quality control included: difficulties in calibrating pipettors, only few thermometers were found to have been calibrated against a certified thermometer.

A review of the global quality assurance programme was reported by D Featherstone. Marked progress in the accreditation of national laboratories since the last meeting was noted. Some laboratory coordinators have recruited other laboratory experts to assist with onsite reviews in order to accommodate the potentially heavy workload. The sample validation accreditation criterion has proven to have logistical, financial and political challenges to some countries and regions. However, evidence was presented of the effectiveness of this criterion in detecting problems in laboratories which were not being detected by other evaluations. The new accreditation checklist was reported to have been helpful and further revision did not appear to be needed. Reporting of sequence data was perceived to be inadequately implemented in many of the regional reference laboratories, though this criterion will be assessed from the start of 2007, 12 months after it was introduced.

Recommendations: Laboratory Quality assurance

*19. The 2006/2007 proficiency testing programme will now encompass all six WHO regions for the first time. To allow comprehensive analysis of the PT results, all laboratories are encouraged to use the Excel reporting spreadsheet and include details of raw absorbances values for antigen and control antigen wells (for indirect assays), positive and negative controls and any calculation factors used to arrive at the final result. All laboratories are required to test all panel samples for both measles and rubella IgM. **Action: All laboratories. Ongoing***

*20. All Regional coordinators are encouraged to collect serum samples suitable for inclusion in the global proficiency programme during any interactions with laboratories such as accreditation visits or during regional or global meetings. The shipping of any serum collections to VIDRL should be coordinated through WHO/HQ. **Action: All Laboratory Coordinators. Ongoing.***

21. *Laboratories should be encouraged to provide complete epidemiological data when shipping clinical samples for validation or molecular epidemiological studies to allow for maximum benefit to be gained from the process. **Action: All laboratories. Ongoing***

Introduction of new techniques

Integrated method for detection of Measles and rubella virus infection in Vero/SLAM cells using an immunocolorimetric assay.

An integrated colorimetric immunoassay developed by CDC to confirm infection of Vero/SLAM cells by measles or rubella virus without the need for expensive fluorescent microscopy equipment showed promising results. The colorimetric protocol was developed for both measles and rubella and sensitivity and specificity was comparable with immunofluorescence techniques. All genotypes were detectable and sensitivity of 1 PFU of rubella virus was possible after three passages in Vero/SLAM. The colorimetric assay was also shown to improve the detection of plaques in rubella PRNT assays.

China and Cote d'Ivoire (CIV) volunteered to evaluate the colorimetric protocol in small field trials. W Xu reported a comprehensive evaluation in three provinces in China, Henan, Jilin and Guangdong. More than 80 samples were tested and showed 100% correlation between RT-PCR and colorimetric detection for 73 measles and 16 rubella cases. In 11 cases RT-PCR and colorimetric assays detected measles virus in the absence of CPE. CIV plan on completing their evaluation by the end of 2006.

Recommendations. Introduction of new techniques

22. *Data from the two field trials of the integrated colorimetric detection techniques for measles and rubella will be evaluated after the CIV study has been completed and a decision made on the introduction into the network as a routine confirmatory testing protocol. **Action: WHO HQ, CDC, CIV and China CDC. First quarter 2007***
23. *China CDC should investigate the underlying cause of the results in the 10 cases in Jilin and one case in Henan where measles virus was detectable by RT-PCR and colorimetric immunoassays but no CPE in Vero/SLAMs was apparent. **Action: China CDC. First quarter 2007***

Expanded Role of the Vaccine Preventable Disease LabNet

Mumps

Mumps surveillance in the UK was reported by K Brown. In 1988 the UK introduced MMR as a single dose at 15-18 month of age and a 2nd dose of MMR was introduced in 1999 for those at 5 years of age. In late 2004 and 2005 a large outbreak of mumps occurred with more than 32,000 suspected cases laboratory tested, 76% of which were had oral fluid samples collected. The predominant genotype in 2005 was G5 in contrast to the G2 genotype in 2003 and early 2004. PCR testing was found to increase diagnosis by >10% in the first 7 days post onset. Collective data for 2003-06 showed ~56% of suspected cases were IgM positive and a further 3% PCR positive and IgM negative. However, more than 16,000 clinical cases were PCR and IgM negative. Suggestions for the large number of IgM negative results were: misdiagnosis, samples collected too early, low sensitivity of assay, infection in previously vaccinated individual, and reinfection with mumps. In a small study (5 cases) where paired sera were collected, rising IgG titres helped confirm mumps infection when IgM was negative.

J Rota reported recent mumps activity in the USA. In late 2005 and early 2006 Iowa and neighbouring states experienced a mumps outbreak mostly affecting the 18-25 age group, many of whom had been vaccinated. By July 2006 more than 4,800 cases had been reported and a G5 genotype, identical to the UK outbreak strain was identified. A reported 54% of cases had been vaccinated with 2 doses of MMR. Some of the diagnostic challenges faced during the USA outbreak were that no commercial mumps assays are FDA approved and much of the attempted diagnosis was being done in private laboratories, using non-validated assays, some of which were shown to have low sensitivity. CDC developed a capture IgM assay and a real time PCR assay for state laboratories' use. From 835 samples collected from 25 states, 100 IgM positive cases were recorded. Some of the low positivity may be explained from the heightened surveillance following publicity of the outbreak and samples collected from non-infected cases. High IgG levels found in some cases may have been due to a booster effect of WT infection following previous vaccination and contributed to IgM being undetectable.

L Jin summarized the collaborative report for standardizing of the nomenclature for mumps virus as published in Archives of Virology in 2005. She also reported the development of a sensitive real-time PCR method for measles, mumps and rubella with sensitivity for mumps of 2-10 copies or 2 pfu/ml.

Japanese Encephalitis

The two WHO regions affected by Japanese encephalitis (JE) described their plans for developing a JE diagnostic LabNet. Both SEARO and WPRO are using the infrastructure of the existing measles and polio LabNet to build laboratory capacity in the regions and will utilize integrated surveillance activities. Development of the JE LabNet will be in conjunction with establishing acute encephalitis syndrome (AES) surveillance, developed jointly through WHO and

Global Disease detection (GDD), CDC. SEARO has developed a plan of phased sentinel surveillance with 13 laboratories being established initially. A training workshop will be held in mid-October where all laboratories will participate in case confirmation using standardized IgM assays. WPRO has plans to establish laboratories in 6 countries China, Vietnam, Philippines, Cambodia, Laos and PNG. A training workshop planned for the end of 2006 has been postponed.

Twinning initiative

S Gognat described the EPR training centre in Lyon contribution to the improvement of the capabilities of resource-limited public health laboratories through the setting-up of twinning projects with specialized institutions. The twinning procedure was launched in April 2006 and has received 84 requests from resource-limited laboratories and 37 offers for support from specialized institutions. The matching and selection of laboratories is being undertaken by a steering committee and will be announced shortly. It is hoped that financial resources will be made available from global or bilateral donors.

Recommendation: Expanded Role of the Vaccine Preventable Disease LabNet

*24. The nomenclature to describe wild-type mumps viruses should follow the recommendations outlined in the collaborative report published in Archives of Virology in 2005. **Action: All Network Laboratories. Ongoing***

*25. Laboratories are encouraged to build on the existing WHO LabNet structure to initiate testing for other vaccine preventable diseases provided that these new activities do not adversely affect ongoing measles/rubella and polio activities. Laboratories are encouraged to pursue additional funding (e.g. GDD and Twinning) to support expansion of testing. **Action: All Network Laboratories and Coordinators. Ongoing***