Summary and Recommendations

Global and Regional Update

The measles and rubella laboratory network (LabNet) consists of 679 laboratories serving 164 countries. A reported 239,617 samples were tested globally for measles IgM in 2008 about 6000 fewer than in 2007. Approximately 80% of the samples were also tested for rubella, resulting in an estimated 430,000 measles and rubella assays being tested in the LabNet for 2008. Timeliness and quality of the testing is high with more than 80% of laboratories reporting at least 80% of their results within 7 days. Of the 168 laboratories to receive the global annual proficiency testing panel in 2008, 98.2% passed the measles component and 94% passed the rubella component. Most of the remaining subnational laboratories participated in national proficiency testing programmes with a similar high level of accuracy.

The WHO African and South East Asian Regions have developed plans for enhanced measles control and the laboratories in these regions will need to respond to an increased workload following the proposed improvement in case based surveillance. Currently, measles surveillance in India requires the collection of a representative 5 samples from each outbreak which in 2008 resulted in 1089 samples being tested by the LabNet. The number of samples required for laboratory confirmation is likely to increase to approximately 23,000 per year once "elimination" quality surveillance in implemented India. Other countries in SEAR are already meeting the expected workload for samples being tested for case confirmation, however improved molecular surveillance will be required to meet the ideal of sequence information gathered from each chain of infection. The African region as a whole tested 16,500 samples in 2008 which equates to just over the recommended surveillance goal of 2/100,000 non-measles, suspected measles cases. However, some countries in the region have yet to reach this goal but the capacity of the LabNet is adequate to meet the expected surveillance improvement in these countries.

Regional Updates

African region

The WHO African region has established a "pre-elimination" measles control goal following early success in meeting their measles mortality goal of 95% reduction in deaths by 2010, based on 2000 baseline data. A strategic framework will be developed to guide the implementation of the pre-elimination target of 2012, including > 80% of districts investigating at least 1 suspected case per year and a non-measles febrile rash illness rate > 2/100,000.

The measles LabNet was established in 1998 and currently consists of 3 regional reference and 42 national laboratories which provide diagnostic support to all 46 member states. In 2008, the measles laboratories completed 28,874 IgM serum tests, more than 90% of which were reported within 7 days of receipt in the lab. Approximately 10% of the serum tests were confirmed at one of the 3 regional reference labs and
showed more than 90% concordance. Measles virus sequencing information was obtained from nine African countries after referral to the sequencing laboratory in South Africa, however there were still many outbreaks where samples for molecular testing were not collected.

The key challenges for the region include: increased workload, ensuring adequate laboratory reagents and equipment, high staff turn over, competing activities for limited resources, national ministries of health providing little support, stock outs of consumables /reagents/kits and equipment failure difficulties in shipping specimens and isolates.

The region has been instrumental in trying to maintain lab capacity by holding regular training workshops with emphasis on standardizing procedures and quality control, and the reinforcing of procedures to encourage virus detection. Experienced staff are encouraged to remain in the lab through salary top ups and there is regular coordination with couriers to overcome shipping challenges.

To meet the needs for enhanced surveillance following the pre elimination phase, AFRO has planned the establishment of additional laboratories, including subnational labs in Ethiopia (3), Nigeria (2), DRC (2), Cameroun (1), and Tanzania (2). Strengthening sequencing capacity in Uganda and CIV is planned and an additional Regional Reference lab is proposed for the Central African region, likely to be Cameroun.

**Eastern Mediterranean Region**

The Eastern Mediterranean region (EMR) has established a measles elimination goal targeted for 2010. All 22 EMR countries have established measles national laboratories for serological confirmation of measles and all passed the global proficiency test. Twenty labs have been fully accredited with Djibouti endeavouring to meet the minimal requirements and Somalia pending evaluation. Seventeen of the labs also have the capacity for virus isolation and detection following virus detection training workshops in 2007 and 2008. Libya, Djibouti, Somalia, UAE and Lebanon have yet to establish virus detection capacity. The Oman and Tunis regional reference laboratories have good sequencing capacity and serve the region, and the Iran, Pakistan, Morocco and Egypt national laboratories also have the sequencing capacity.

EMR has more than a third of their countries which are close to elimination and the region is developing measles elimination validation guidelines to help countries confirm their success. Laboratory quality control is a critical part of this process and EMRO has strengthened monitoring QA/QC in the region through shipping serum samples dried onto filter paper for confirmatory testing. Iran, Egypt, Morocco, Sudan and Syria have all used this technique to obtain concordant results. However, two countries: Saudi Arabia and UAE have yet to participate in the external quality control programme.

Drying virus infected culture material onto filter paper has also been successfully used in the region for shipping viruses between national and reference labs without the need of cold chain.

Challenges for the EMR LabNet include;

- Meeting the increased laboratory workload as surveillance improves
- Ensuring H1N1 activities do not impact negatively on measles and rubella surveillance
- Measles validation process
• Assuring quality of non-validated IgM assays
• Improving virological surveillance
• Finding sufficient external resources for laboratory supplies and training
• Encouraging countries to integrate lab testing costs into their surveillance programme budget
• Encouraging countries to focus more attention on rubella and CRS surveillance

European Region

The European region has seen a marked reduction in the number of measles cases in 2008 compared with previous years but outbreaks are still occurring in countries mainly in western Europe. Almost 90% of the 4301 measles cases detected in 2009 were found in countries representing 25% of the region’s population. The majority of outbreaks are in Western Europe with 82% of cases found in unvaccinated individuals and 38% in over 15 years of age.

The LabNet comprises 70 laboratories, including 20 sub-national laboratories, three RRLs and one Global Specialized laboratory. Laboratories tested 25426 IgM samples in 2008, 3000 fewer than 2007 due to the reduction in measles incidence. Laboratories reported 80% of the test results within the required 7 days. However, Italy is not reporting any data and Turkey has stopped reporting.

All labs in the region except two (Bosnia and Herzegovina and Malta) underwent desk accreditation assessments in 2008 and all were determined to be fully accredited except Kyrgyzstan which was provisionally accredited. Seventy three labs participated in the WHO Global Proficiency testing programme in 2008, including subnational labs in the Russian Federation, Turkey and Ukraine. Proficiency was excellent with only 1 laboratory failing to achieve a passing score.

Russians Federation labs have started using locally produced kits and most other labs are provided with kits by their ministries, with only a small number of countries receiving kits from WHO.

Key challenges identified for the EUR LabNet were;
• Improving M/R data quality in the Region
• Strengthening communication within surveillance teams to ensure linking epi and lab data
• Identifying a clear mechanism of laboratory reporting to a country focal person for Measles/Rubella surveillance (or an EPI manager in selected countries) in all Member States and to WHO
• National laboratories collaborate with the private and University diagnostic labs to ensure high quality and complete reporting of measles and rubella data, including genotyping information
• Address and resolve methodological challenges in achieving elimination, especially related to low PPV, false positive IgM and consideration of the wider use of PCR; automated testing systems, and alternative sampling procedures
• Plans to work on implementing OF and DBS where this is warranted
• Develop sera panels for validation purposes
Region of the Americas

The Region of the Americas’ (AMR) LabNet is made up of 148 laboratories of which 124 are sub-national, 21 national, 2 regional reference and 1 global specialized. A total of 45,808 samples were tested in the region in 2008. Labs confirmed 207 measles cases from sporadic cases in a small number of countries and 3730 rubella cases. The LabNet operates at high a level of performance with all national laboratories passing the WHO global proficiency test and 86% of samples reported within 4 days of being received in the laboratory.

The PAHO Measles and Rubella Laboratory Network meeting in 2009 focused on the challenges facing the region, having eliminated measles and being close to eliminating rubella. The meeting recommended increasing laboratory’s sensitivity in confirming sporadic cases, improving molecular epidemiology information, and collection of additional samples for additional tests.

South East Asian Region

The South East Asian region has established a measles goal of 90% mortality reduction by 2010. All countries except India have implemented case based surveillance and are reporting aggregated data to the SEARO monthly. India is currently conducting outbreak investigation in 6 states and has plans to expand to more.

All countries have established measles laboratories with Timor Leste's laboratory now fully operational. India has established 5 labs to cover states which have initiated surveillance and an additional 2 laboratories supporting reference activities, NIV Pune for molecular purposes and Chennai for serological testing. A further 7 state labs are planned for 2010-2011 with priority of new labs to be established in the states of Rajasthan and Andhra Pradesh early 2010. Jakarta serves as a reference laboratory for the 3 Indonesian labs and the regional reference laboratory in Bangkok serves the remaining SEAR countries for serological and molecular reference purposes. In 2008 a regional virologist’s meeting and intercountry laboratory training was conducted.

Eighteen labs 18 Laboratories have been accredited with NIV Pune pending. Baseline measles genotype data is available for all countries except Bhutan and Timor Leste. Rubella baseline data has been reported for Bangladesh, India, Nepal, Thailand and Sri Lanka.

It is anticipated that if the ten countries implementing case based surveillance reach expected performance indicators and India implements their planned surveillance expansions then serum samples tested could rise from the 5000 in 2009 to 15000 in 2010. In addition, it is anticipated that approximately 100 viruses will be sequenced. Sri Lanka is planning on introducing oral fluid devices as a mechanism to improve surveillance in the country. Currently there is some resistance to venipuncture and it is a challenge to collect samples from all suspected measles or rubella cases. Training of laboratory and surveillance staff is planned for 2010.

Western Pacific Region

The Western Pacific region (WPR) LabNet comprises 16 National laboratories, 3 regional reference laboratories and 1 global specialized laboratory. In addition China has established a network of 31 provincial and 331 prefecture laboratories which follow
the WHO standardized testing and reporting structure and have established a strong QA programme. The region has made good progress in moving towards achieving the measles elimination goal of 2012. All countries conduct case based and laboratory supported measles surveillance. Surveillance performance is improving with completeness of reporting rising from 51% in 2007 to 78% in 2009, and timeliness of reporting from 19% to 57% over the same period.

In 2008, 130,580 serum samples were tested for measles IgM in the region, 121,000 from China. However, reporting of measles data to the regional office is not optimal, with 6 countries not reporting laboratory data including 2 key reporting countries (China and Japan). All China provincial labs are now trained in performing both IgM and RT-PCR techniques for measles and rubella.

All national labs except for PNG were reviewed and accredited, with PNG planned to be reviewed in late 2009. All 31 Provincial labs in China will become part of the global PT testing programme in 2009. Prior to 2009 the provincial labs tested an annual PT panel compiled by the China CDC. Japan has introduced case based surveillance for all measles cases from 2008 and established 10 measles reference centres using ELISA and the Global laboratory at NIID, Japan has implemented PT and confirmatory testing. Regular communications has been established with all countries except for PNG and the PICs. A confirmatory testing mechanism was established in most national labs by 2009.

- The challenges and plans for 2010 include full implementation of timely, case-based laboratory reporting in those countries where it is not occurring.
- Implementing ELISA QC of commercial labs in countries where these are a predominant testing e.g Japan
- Improved communication with PIC and PNG labs
- Data management

**Molecular surveillance**

The tracking of virus globally can help determine whether outbreaks are caused by endemic or imported virus strains and can monitor progress with achieving measles control goals. There has been a marked improvement in the collection of measles and rubella molecular epidemiological data as more laboratories develop capacity for molecular techniques and the programmatic value in tracking viruses has been recognized. In 2006 a WHO genotype database was established to track measles viruses detected by the LabNet. By October 2009, genotype information from 7096 measles viruses had been submitted to the database comprising representatives of all 23 genotypes from 117 countries with viruses dating back to 1954. Approximately 34% of these viruses have also had sequence information submitted to GenBank. For rubella, 465 viruses have been submitted, representing all 11 genotypes from 38 countries, with 48% of them with sequence data submitted to GenBank.

Genotype B3 was the predominant genotype found in Africa in 2008 and 2009. Countries with B3 genotype included Burkina Faso (>50,000 cases and 300 deaths), Cameroon, (with evidence of multiple importations), South Africa which experienced a large outbreak in Gauteng Province in 2009 with B3 virus identical to 2008 Benin B3...
viruses. Other countries with B3 detected include; Benin, Cote d'Ivoire, DR Congo, Equatorial Guinea, Ethiopia, Mali, Niger, Togo and Uganda. Mali's B3 viruses were distinct from other B3s found in the region.

One case in South Africa was detected with D8 virus which had epidemiological and virological links to India.

The Eastern Mediterranean region reported large measles outbreaks in Egypt, Iraq and Saudi Arabia in 2008 and in Iraq (>12,000 cases) in 2009. The predominant genotype found in the region continues to be D4, (Afghanistan, Egypt, Iran, Iraq, Morocco, Oman, Pakistan and Sudan). B3 was found in outbreaks in Libya and sporadic cases in Tunisia in 2009 and D8 was identified in Kuwait, Libya, Morocco and Oman.

In the South East Asian Region, only 2 countries reported genotype information in 2008 and 2009. India reported D4 and D8 viruses from their molecular surveillance programme coordinated by NIV, Pune and the Thailand RRL in Bangkok reported D5 and D9 viruses identified from outbreaks in Thailand.

The Western Pacific region reported genotype data from Australia (D4, D5, D8, D9, H1) China (H1, D4, D9), Hong Kong SAR (H1), Japan (D5, D8, D9, H1), Malaysia (D9), New Zealand (D9, H1) and Singapore (D4, D5, D8, D9, H1) over the period 2008 and 2009. Interestingly China reported two cases where non H1 viruses were detected and subsequently found to have epi-links to France (D4) and Thailand (D9). Both cases were thoroughly investigated and no evidence of spread was found in contacts. Australia reported H1 virus in cases with epi-links to Iran. Iran subsequently found H1 cases with epi-links to workers from China.

In the European region a large outbreak of measles in the UK occurred between 2007 and 2009 where sequence analysis confirmed identical sequence in the majority of cases which was identified as D4, and labelled after the first case identified, MVs/Enfield.GBR/14.07. Over 3,000 people within UK have been infected with same strain and at least 1,500 people in rest of Europe. An identical virus has been exported to at least 18 other countries, mostly in Europe but was also found in the Eastern Mediterranean region and the Americas. The Enfield strain has been found to have closest links to an Indian strain from 2005, with 3 nt difference. The UK reported that the Enfield strain stopped circulating in December 2008 except for an importation in March from Italy.

The Luxemburg RRL reported that six hundred measles cases were notified by France in 2008 and more than 1000 cases in 2009. D4, D5, D8, D9 and H1 viruses were identified from samples tested by the national laboratory in France in 2009 including a D5 virus which spread from a school outbreak just prior to the holidays and spread over most of the country.

Robert Koch Institute reported that Switzerland experienced 3 waves of measles cases since November 2006. D5 "Lucerne" virus made up the majority of the first 2 waves (2007 and 2008) and part of the 3rd (2009) wave with D4 Enfield and B3 virus also being identified in the 1149 cases identified in 2009. The D5 "Lucerne" strain also spread from Switzerland to Germany.

It was reported that some countries have multiple importations with the same virus genotype but without any epi-link found, stressing the importance of collecting
representative clinical samples for sequencing which allows determination of whether viruses are imported or endemic.

There are still information gaps in molecular surveillance in some countries, but these are gradually being filled as training workshops build laboratory capacity and awareness of the need for molecular surveillance occurs.

Merck has provided funding for enhancing measles surveillance in AFR and 5 pilot countries (Malawi, Zimbabwe, Kenya, Benin and Cote d'Ivoire) will utilize oral fluid sampling devices for collecting samples for both case confirmation and molecular surveillance.
Surveillance of molecular diversity of measles and rubella

1. Molecular data obtained from outbreaks and sporadic cases of measles and rubella, in combination with epidemiological information, can provide useful information on the likely sources of infection and differentiate between indigenous and imported viral strains. The WHO measles and rubella genotype database and MeaNS sequence databases are functioning well and are used by almost all of the sequencing laboratories in the LabNet. These databases are proving to be valuable tools for facilitating molecular surveillance, however;
   a. Efforts should be made to collect molecular surveillance data from all chains of infection and especially in those countries where no baseline sequence data exists.
   b. LabNet should utilize the well validated tools and samples available for enhancing molecular surveillance where appropriate, such as;
      i. Oral fluid, throat swabs, urine and PBMC as samples for virus detection
      ii. Detection of viral RNA in archival sera
      iii. Shipping of samples dried onto filter paper
      iv. Conventional and real time PCR for case classification and molecular surveillance

   **Action:** LabNet. **Timeline:** Ongoing

2. Laboratories are reminded of their requirement to submit representative genotype information on their measles and rubella strains to the WHO genotype database, preferably on a real-time basis, but at least by the end of the month the genotyping was completed. **Action:** LabNet. **Timeline:** Ongoing

3. The HPA MeaNS measles sequence database now has the capacity to be used globally. It is recommended that:
   a. All countries are encouraged to submit measles sequence data to the HPA MeaNS measles sequence database following the WHO recommended timeliness indicators. Subnational level laboratories with sequencing capacity should submit data to their national laboratory for validation and submission.
   b. National laboratories with sequencing capacity should consult with their designated Regional or Global sequencing laboratory before submitting their sequences to the database to assure the quality of the data submitted.
   c. Regional sequencing laboratories should report data from their national measles and rubella surveillance programmes and should strongly encourage countries for which they provide sequencing support to report their own data or obtain written permission from them to submit the data on their behalf. Regional sequencing laboratories can encourage countries to report their own data by providing sequence data in a format compatible with the LabNet database needs and by assisting with preparation of MeaNS or GenBank entries.
d. HPA is encouraged to develop a similar global database for rubella and incorporating phylogenetic analysis tools. Until this has been established, rubella genotype data should continue to be submitted to the WHO genotype database.

e. A small informal "steering committee" will be formed to review protocols and address ongoing issues with the sequence and genotype databases.

**Action:** All LabNet: **Timeline** Ongoing.

**Action:** Steering Committee (WHO/GSLs/RRLs) **Timeline:** First quarter 2010

4. To increase the value of sequencing information, key epidemiological information including; date of sample collection, date of rash onset, date of vaccination, unique identifiers, case location, travel history and epi-link to other cases should be provided to the network laboratories through their country surveillance teams. **Action:** WHO, LabNet. **Timeline:** Ongoing

5. Sequencing laboratories are requested to share representative virus isolates with the designated strain banks for reference purposes. **Action:** LabNet. **Timeline:** Ongoing

6. Countries without baseline rubella sequencing information should consider utilizing archival sera for rubella genotyping purposes. The regional laboratory coordinator should be consulted as to the appropriate procedures and site for testing these samples. **Action:** LabNet. **Timeline:** Ongoing

**Quality assurance**

7. A standard protocol has been developed for using serum dried onto filter paper for confirmatory testing purposes. This has been evaluated under "field" conditions in the EMR and confirmed earlier evaluation results showing good concordance with liquid serum. It is recommended that regions consider introducing this technique where challenges in shipping samples to reference laboratories exist. To ensure that a gold standard reference is always available, the corresponding liquid sera should be stored at the submitting lab until confirmatory testing has been completed. **Action:** LabNet. **Timeline:** Ongoing

8. National laboratories should store measles and rubella serum samples at -20°C or lower for use as; internal controls, for global proficiency test panels and for virus identification. Those laboratories with stocks of positive samples (at least 0.5 ml and preferably volumes approximating 10ml) are requested to contact their Regional laboratory coordinator to facilitate using these samples in the WHO proficiency and QC programme. **Action:** WHO LabNet. **Timeline:** Ongoing.

9. Building on the European WHO and EU initiatives the LabNet will develop a questionnaire to identify the range and scale of molecular testing within the LabNet as a preliminary step to developing a molecular QA programme. A trouble shooting guide for PCR, sequencing and analysis will also be developed. Establishing and distributing "run controls" are planned for 2010. **Action:** HPA, CDC, WHO. **Timeline:** July 2010
10. The LabNet will establish a protocol and trouble shooting guide for ensuring optimal cell sensitivity testing for the isolation for measles and rubella viruses in Vero/SLAM cells. **Action:** GSLs, WHO. **Timeline:** July 2010

11. The volume of proficiency testing sera will be increased for those laboratories using automated IgM testing systems and consequently require larger volumes than currently distributed in the LabNet. **Action:** VIDRL, EUR LabNet. **Timeline:** Aug 2010

12. A more comprehensive analysis of national laboratories proficiency test results should be carried out by the regional lab coordinators to determine trends which may help identify testing issues. **Action:** WHO Coordinators. **Timeline:** Ongoing

13. Rubella virus detection methods described in the WHO measles and rubella laboratory network protocols need to be implemented more widely, including ensuring that network laboratories have access to high quality colorimetric reagents, primers and RNA controls. **Action:** CDC, WHO LabNet. **Timeline:** Ongoing

14. As countries approach the elimination stage and most samples are negative for IgM, the PPV for confirmatory testing decreases. Therefore, laboratories should ensure that systematic internal quality control procedures are performed to ensure accuracy of serological testing. LabNet will develop a guideline for establishing and using in-house quality control methods to monitor accuracy of assays and the performance of the laboratory. **Action:** HPA, CDC, WHO. **Timeline:** July 2010

15. In countries where most measles and rubella IgM testing is performed in private/commercial laboratories, it is vital that the performance of these laboratories is monitored. Performance of these laboratories should be assessed through an external quality assurance programme and pre-existing quality assurance data should be collected by the national laboratories, where possible. These private laboratories are encouraged to share their measles and rubella data with the relevant national reference laboratory on a regular basis. **Action:** LabNet. **Timeline:** Ongoing

16. Laboratory and surveillance colleagues are encouraged to meet regularly to ensure that laboratory and surveillance data are harmonized. **Action:** LabNet. **Timeline:** Ongoing

**Coordination**

17. LabNet is concerned about the number of laboratory coordinator vacancies currently unfilled or that will become vacant in the near future. LabNet members are encouraged to apply for these positions and/or share the vacancy descriptions with potential candidates. **Action:** LabNet. **Timeline:** October-December 2009

**Communication**
18. A regular communication link between working sub-groups of LabNet members should be established to facilitate development of protocols and procedures for the LabNet. The key issues requiring development and deployment include;
   a. IgG and IgM sera panels for validating serosurveys and for evaluating the non-validated IgM assays used in a small number of countries
   b. Classifying sub-genotypes of rubella (specifically genotypes 2B, 1G and 1E) for the specific purpose of molecular epidemiology
   c. Virus culture sensitivity evaluation and trouble shooting protocols
   d. Internal QC protocol
   e. QA/QC procedures and trouble shooting guides for virus isolation and molecular techniques.

**Action:** HPA, CDC, WHO, LabNet. **Timeline:** Quarterly teleconferences

**Enhanced Surveillance**

19. Serosurveys have been demonstrated by some countries to be a useful tool for determining population immunity, especially where other data, such as laboratory based surveillance and vaccine coverage, may be limited or suspect. To validate the serosurveys a serum validation panel should be developed along the lines of the ESEN study. A protocol / guideline for the implementation, validation and interpretation of serosurveys will be developed. **Action:** HPA, CDC, Luxembourg, LabNet WHO. **Timeline:** July 2010

20. Point of care (POC) rapid test systems have the potential to rapidly identify outbreaks/cases. The measles POC assay data presented by HPA shows promising sensitivity and specificity compared to conventional IgM ELISA under lab conditions. The LabNet encourages the further development of POC assays and recommends evaluating these under field situations. **Action:** HPA, CDC WHO LabNet. **Timeline:** December 2010

21. Several WHO regions are developing protocols and indicators to document elimination of measles and/or rubella. Regional Laboratory coordinators should share laboratory-specific components of these protocols with other LabNet members for feedback and to ensure consistency within the LabNet. PAHO should share the laboratory components of their document with the LabNet before the next LabNet global meeting. **Action:** WHO, LabNet. **Timeline:** Ongoing

22. The principle of drying reagents, sera and viruses onto filter paper has been validated in reference laboratories and in the field. Laboratories should consider using this technique, following the prescribed protocols, to ship samples where appropriate. **Action:** LabNet. **Timeline:** Ongoing

**Advocacy**

23. To ensure the LabNet has sufficient capacity to meet the enhanced laboratory-based surveillance activities in alignment with AFRO and SEARO's pre-elimination and mortality reduction goals, the regional coordinators are requested to identify their needs for resources and support for training activities.
for the next biennium.  **Action:** WHO AFRO, SEARO and HQ.  **Timeline:** Ongoing

24. The LabNet should investigate strategic pairings with other laboratory based surveillance programmes (such as HIV, Flu, Polio) **Action:** LabNet WHO.  **Timeline:** Ongoing

25. The LabNet endorses the Laboratory related topics proposed for the forthcoming JID review supplement and are prepared to meet the publication deadlines. **Action:** LabNet.  **Timeline:** Table of contents finalized by November 2009, chapters submitted 2nd quarter 2010.