Report on the
9th WHO Global Measles Rubella Laboratory Network Meeting
WHO Headquarters, Geneva, Switzerland
19-21 September 2011

Rapporteurs: J Leydon, Dr J Goodson

Introduction

The WHO Global Measles and Rubella Laboratory Network performs a key role in measles and rubella surveillance by confirming suspected cases using standardized and validated testing and reporting procedures. The WHO Measles and Rubella Laboratory Network (LabNet) comprises 690 laboratories globally, almost all of which are following standardized testing and reporting procedures and undergo regular quality assurance and proficiency testing assessments. Representatives from key specialized and reference laboratories within the LabNet and the WHO laboratory staff responsible for the coordination of the LabNet meet annually in Geneva. In 2011, the meeting discussed in 8 sessions the progress within the network, measles surveillance, tracing measles virus transmission, proposals for improving surveillance and enhancing surveillance, validation and implementation of new technologies, strengthening quality assurance and standardization, data management and the challenges of meeting elimination criteria for measles and rubella.

Meeting objectives

The objectives of the meeting were as follows:

1. To review and discuss the current status and management of the Global Measles LabNet in order to develop and strengthen the technical capacity and structure of the network.
2. To provide WHO staff and Measles and Rubella LabNet representatives with technical updates on the laboratory issues related to measles and rubella control.
3. To determine how best to meet future challenges for the measles and rubella network.

Expected outcomes:

Technical recommendations and plan of action for further development and strengthening of the measles and rubella LabNet for 2011-2012.

Session 1: Opening

In his welcome speech Dr. Jean-Marie Okwo Bele, director of the WHO Department of Immunization, Vaccines and Biologicals, highlighted the recent progress made in the Global Measles/Rubella Laboratory Network. The laboratory network plays a key role in achieving measles elimination, it provides accurate and timely data on measles case confirmation, conducts differential diagnosis, provides data on rubella surveillance, and provides the evidence on the source of the virus that is causing outbreaks. It has become evident that molecular surveillance and rapid sharing of data is critical to understand indigenous circulation or importation of measles viruses. The meeting provides a means to share surveillance and molecular data, discuss and roll out new diagnostic procedures and molecular technologies.

Exciting developments such as a DOV launched by Bill Gates at the World Health Assembly (WHA) in 2011 and an action plan for measles are being introduced. The WHA asked WHO to work on an action plan, the initial draft to be finalized next week in Barcelona.

The GAVI replenishment meeting in London in June 2011 was very successful. There will be funding for new vaccine introduction. There is a growing realization that measles control is central to VPD.
efforts. Recent funding support from NORAD and DFID has provided welcome additional support for measles control efforts.

**Session 2: Global & Regional Briefings**

An update on the global Measles and Rubella elimination strategy was provided by Dr. Peter Strebel, who leads the Global Measles Control Programme at WHO/HQ. All Regions now have measles control goals, AMR succeeded in both Measles and Rubella elimination by 2010, EUR has set an elimination goal by 2015, AFR by 2020, EMR by 2015, WPR by 2012 and the South-East Asian Region endorsed the 2015 mortality reduction goal but has yet to set a final elimination date.

The global goal initiated in May 2010 is for 90% of vaccine coverage at a national level and an 80% coverage in every district and for the reported incidence to be <5 cases of measles per million.

By July 2011 one billion people have been vaccinated, the reported number of cases is down by two thirds and the number of deaths has decreased by three quarters.

Countries should be encouraged to vaccinate for rubella taking the opportunity offered by the measles immunization activities and the combined vaccines. The minimum average coverage required is 80% or greater but the most recent change in the recently published rubella position paper is that the 80% minimum coverage can also be provided by SIAIs rather than only routine services. It is recommended that CRS surveillance is introduced to monitor progress towards rubella control.

A global strategic plan document 2011-2020 is being drafted and will be shared with the LabNet. A reduction in mortality >95% is scheduled for 2015 with regional measles and rubella elimination by 2020. Five WHO regions have measles elimination goals. Measles is most infectious and most visible so can be useful for identifying gaps in the program and synergies with other VPD efforts.

**Strategies to be implemented:**

- High vaccine coverage- 2 doses
- Outbreak preparedness and response
- Research and development

An update on the Global Measles/Rubella laboratory network was provided by David Featherstone, Global VPD Laboratory Network coordinator. The number of laboratories within the network totals 690 and 183 Member States are served by a proficient laboratory. All laboratories passed the WHO proficiency testing programme, with most achieving a 100% score.

A number of new procedures are in the process of being validated including an RT-PCR EQA programme for measles and rubella, new synthetic positive PCR controls and rapid point of care assays.

It was proposed that labs use the WHO Genotype SharePoint to reference newly updated laboratory procedures rather than continually updating the laboratory manual.

The recently published JID Measles and Rubella supplement had 10 laboratory related papers and was a useful monitor of progress with the development of the WHO measles/rubella LabNet.

Monitoring laboratory performance indicators needs to be updated, with changes in the laboratory accreditation checklist especially related to the reference role that National laboratories have which support sub-national labs in their countries.

Regular data reporting to WHO is progressing and global data is reported monthly, however further harmonization of laboratory based reports and the epi-based laboratory reporting is needed.

**Challenges for the network are:**

- Rapid detection of measles cases
- IgM detection alone in the elimination phase may not be sufficient.
- The PPV of IgM testing is lower in low incidence settings.
- Enhancing molecular testing, especially in the countries with no baseline data
- Proof of quality of laboratory is required for verification of elimination.
Regional LabNet updates; progress and challenges

AFR- Dr Annick Dosseh

There are 44 laboratories in the African region, one laboratory per country for most countries except Nigeria which has 4 laboratories and Burkina Faso which has separate measles and yellow fever labs. Capacity building has been improved by a training course in measles/rubella genotyping and sequencing held at CDC, Atlanta for participants from Uganda. There has been an increase in the number of countries meeting the performance indicator of <10% of measles IgM positive cases in 2011 compared with 2010. Overall laboratory timeliness of reporting within 7 days was >80% in 2011 though 4 labs are reporting <50% of results within 7 days. The underperforming laboratories had either a measles outbreak or shortage of kits which delayed testing. Most labs in the AFR network are performing adequately with 31 accredited, 3 provisionally and 5 labs with accreditation pending. Genotypes identified in 2011 were B2 and B3, with B3 predominating and found in most countries of the region. Most of the molecular surveillance data is gathered from countries serum samples sent to NICD in South Africa for confirmatory testing. However surveillance gaps still occur. Many challenges remain, including: lack of backup staff, shortage of measles kits and some funding gaps still occur. Kit shortage often occurred in countries with big outbreaks where no reduction in the frequency of sample collection occurred. Future plans include strengthening capacity for molecular surveillance, developing a national contingency plan for procurement of measles/rubella kits and strengthening resource mobilization.

EMR- Dr Hinda Ahmed

The major challenge in the region has been the political upheaval occurring in several of the countries. The measles elimination target has been postponed until 2015. A number of countries have achieved > 95% vaccine coverage, however there are still pockets within these countries where low coverage can be found. Jordan, Palestine, Syria and Tunisia have reported zero measles cases for the past 3 years. A regional Validation Committee has been formed with nine international experts and six countries have nominated National Validation Committees. All countries passed the proficiency panel, however some laboratories were delayed in their reporting, due mainly to shortage of kits. All laboratories demonstrated good concordance with their serum testing. Filter paper with dried serum spots was the preferred method for sending the samples to the RRL. Eighteen of the 23 laboratories in the region have molecular testing capacity. Libya and Somalia are without. Four laboratory staff underwent training for RT-PCR and 5 had serology training and countries achieved good results for the molecular performance test following the workshop. No molecular reports from Lebanon, UAE and Bahrain. A new Laboratory has been built in the new country of South Sudan and will be testing shortly. All NMLs are fully accredited except for two which are provisionally accredited. Challenges include frequent staff turnover in key labs and ensuring RRLs get all surveillance data for identifying cases and speed reporting to the sequence databases.

EUR-Dr Mick Mulders

The European Region has made a renewed commitment to measles and rubella elimination and CRS control by 2015. The strategy to achieve this goal focuses on ensuring high vaccination coverage of 95% for 1st and 2nd dose measles and 1-dose rubella, and strengthening measles, rubella and CRS surveillance. Vaccination coverage was reported sufficiently high as >95% with two measles doses being received. However, susceptible populations continue to be affected, predominantly young adults and students, health care workers and ethnic groups like Roma or Travellers. As a result, large measles outbreaks occurred in Bulgaria in 2010 and in France in 2010-2011 with >20,000 cases reported and 6 deaths. The challenges are maintaining high routine immunization coverage and
reaching the hard-to-reach and marginalised populations and that Rubella is low on the agenda in many of the European countries.

Western Europe had the most measles outbreaks. Measles in France was mainly genotype D4. For 2011, other reported genotypes in Europe were: B3, D8, D9, G3 and H1.

The WHO/European measles-rubella laboratory network consists of a total of 71 laboratories, 66 of which underwent the annual accreditation review in 2011 conducted by the regional LC. All were accredited.

The role of the laboratories is becoming increasingly important with the elimination goal approaching. The EURO surveillance guidelines define several performance indicators for laboratory. 1) Laboratory confirmation rate should be >80% of all clinical cases. However, member states are facing challenges in terms of reporting suspect cases, obtaining data from private laboratories, obtaining samples, but also linking laboratory and epidemiological data. Alternative sampling techniques have proven in several member states to solve some of these aforementioned issues. Efforts are underway to strengthen ways to link epidemiological and laboratory reporting. 2) Furthermore, at least 90% of chains of transmission should have genotype information. Although member states are increasing efforts to provide genotype information, the 90% threshold still has to be reached. Collecting appropriate samples remains a challenge. Also, reporting outbreak data and integrated epidemiological and laboratory data is challenging. Importantly, however, molecular typing has become an integrated tool in the routine surveillance of measles, and increasingly also rubella. 3) When the incidence drops below 1/1,000,000 the detection rate of suspected measles should be at least 2/100,000 to ensure adequate performance of the national surveillance system.

WHO/EUR LabNet is facing increased workload due to the ongoing outbreaks in the region with more samples investigated by September 2011, compared to the whole 2010. In terms of performance, laboratories are performing well. Looking at the confirmatory test results, 51/67 achieved 100% concordance for measles and 45/67 achieved 100% concordance for rubella. Only one laboratory had <90% concordance for rubella. The proficiency testing results were good with 64/67 having a score of 100% for measles and 61/67 a score of 100% for rubella. Laboratories are gradually improving their completeness and timeliness of reporting data to their national programme and WHO.

There are plans for an RRL meeting in the winter of 2012 and a molecular workshop in the spring or summer. EUR LabNet continues to face challenges as it is a diverse and challenging region, and with limited funding to implement molecular surveillance, implementation of case-based surveillance, and documenting elimination, a process already started in anticipation of the 2015 deadline. The role of the laboratories to provide evidence to support completion of essential elimination criteria should be clear, as molecular epidemiological analysis is critical, but also laboratory-based surveillance. The laboratory is critical for adding specificity to surveillance information, particularly with declining incidence and monitoring progress towards elimination.

WPR-Dr Youngmee Jee

The elimination goal for the Western Pacific Region is 2012. There has been a large measles outbreak in the Philippines in 2010-2011 and an increased incidence in New Zealand and Malaysia in 2011 but overall the incidence of measles has decreased. Greater than 100 million were vaccinated in China in late 2010. In 2011, the number of measles cases decreased in Viet Nam but rubella outbreaks continued in Viet Nam and in the Philippines. Small rubella outbreak cases were detected in PNG and Fiji, and Cambodia also detected increased number of rubella cases in 2011.

Out of 382 network laboratories in the region, WHO conducts laboratory accreditation of 48 network laboratories including 31 provincial laboratories in China. Forty-seven of the 48 laboratories in the region are accredited.

To ensure the quality of routine testing of network laboratories, confirmatory testing has been performed by the RRLs in Hong Kong and Australia with a concordance rate of >90% and most laboratories achieving 100%. For the 2010 PT panel, 48 laboratories scored 100% for measles and 39/48 scored 100% for rubella, 7 scored 95% and one scored 90%.
WPRO receives laboratory data (both aggregated and line-listed) from network laboratories on a monthly basis. Completeness and timeliness of reporting was 92.4% and 82.6% respectively, with the majority achieving 100%.

The main genotypes in the region were D9 from the Philippines and Cambodia, and H1 from China. Timeliness of reporting IgM results within 7 days in 2010 was 45%, due mainly to kit shortages. Laboratory testing data from China, which represents 84% of the regional data, is not included since the country has yet to officially report laboratory data to WPRO.

Plans for 2012 include: strengthening molecular surveillance for measles and rubella in all countries including Philippines which will enhance strengthen virus surveillance after SIA in 2011, and a training workshop to be held in Hong Kong in 2012.

The main challenges for the future are minimizing the impact of budget constraints and the successful integration of laboratory and epi data.

AMR-Dr Marilda Siqueira

AMR has achieved elimination of measles and rubella. There are 148 laboratories in the network, 21 National laboratories, 2 RRLs, 1 Global specialized laboratory plus 124 sub-national laboratories. The network tests 30,000 to 40,000 serum samples per year.

The main challenges are now case classification and interpretation. Additional tests such as IgG and avidity tests need to be fully integrated into the regional network.

If there is a sporadic IgM positive case additional samples are needed for confirmation. A sample for genotyping is also requested.

All laboratories in the region passed the PT panel and achieved a rate of 90% for reconfirmation of serological results. A LabNet meeting was held in June 2011 at CDC and the main recommendations were presented.

The main challenges are to have a dedicated regional laboratory coordinator for the Americas appointed as soon as possible and the considerable requirement of achieving the laboratory related issues leading to the documentation for verification of measles and rubella elimination. This is due by end of 2011 in time for submission to PAHO's Directing Council in early 2012.

SEAR- Dr Nalini Ramamurty

The SEA region has not set an elimination goal yet. The current goal is achieving MCV1 coverage >90% and > 80% in all districts, and reducing measles incidence to <5 cases per million. Bhutan, DPRK, Maldives, Sri Lanka, and Thailand achieved National MVC 1 coverage of >95%.

Regionally, 90% reduction in mortality was achieved by 2010 compared to 2000. Second dose MCV is being introduced either through routine immunization or through supplementary immunization campaigns.

There are 22 laboratories in the region, 19 National Laboratories, 2 National Reference laboratories in India and 1 RRL in Thailand.

Timeliness of reporting in 2010 was 71% which had been impacted by shortage of kits and by labs trying to save on strips. Labs were advised to focus on timeliness rather than economizing and in 2011 timeliness improved and met the >80% goal.

Twenty laboratories participated in the global proficiency test, 18 scored 100% for measles and rubella and 2 scored 95%. Except for the two new laboratories, 20 laboratories have been accredited.

Virus detection is an area being strengthened with 15 laboratories having established virus isolation or virus detection capacity. Three laboratories, the Regional reference laboratory in Bangkok, the National Reference laboratory in Pune, India and the National laboratory in Bandung, Indonesia provide sequencing and genotyping support. Sequence and genotyping data is being submitted to the WHO genotype database and MeaNS database, however timeliness and completeness needs to be further strengthened. Indonesia has yet to report sequence data to the programme. Measles genotypes: D4, D5, D7, D8, D9, G2, G3 and Rubella genotypes 1E and 2B have been reported.
At this point of time there is adequate capacity in the countries for serology and virus detection, however additional laboratories are being considered, especially in India, to meet the increased workload once national case based surveillance is established. Pilot testing of oral fluid for measles and rubella surveillance (IgM) is being performed and virus detection in oral fluid is ongoing.

The main challenge is linking the laboratory and surveillance data, which is being addressed.

Dr Ramamurty officially retired at the end of August 2011 and the SEAR LabNet will supported by Dr Jagadish Despande in the future. The LabNet was highly appreciative of the endless energy and enthusiasm shown by Dr Ramamurty in building and supporting the LabNet in SEAR and wishes her all the very best of luck for her retirement.

Session 3: Measles Surveillance: Tracing measles virus transmission pathways, identifying gaps and proposals for improving surveillance performance

Ms Sheilagh Smit reported on the recent measles outbreaks in South Africa. From 2009-2011 18,440 measles cases tested IgM positive, covering 9 provinces. Of 17,539 cases, 52% were under 5 years, 35% under 1 year and 8% under 9 months. Sequencing data for the AFR southern and eastern block of countries was mainly obtained by nested RT-PCR testing of acute measles IgM positive sera sent for confirmatory testing. Outbreaks in southern Africa were mostly related to the South African outbreak B3 strain. In the rest of Sub-Saharan Africa, measles was widespread and genotype B3 predominated. Rwanda and Uganda detected a B3 virus strain 11-12 nt different to the predominant South African (Pretoria) strain. Angola found B2, and Namibia B2 and B3. Oral fluid samples from CIV were all B3, but showed considerable variability as did the B3 viruses from Nigeria.

Dr Suleiman Al Busaidy presented measles genotyping data for EMR. In 2010 and 2011, B3 and D4 predominated in the region. In 2010, B3 was found in Oman, B3 (also D4), Yemen, Saudi Arabia (also D4 & D8) and Somalia. Genotype D4 was found in Pakistan, Iran and Qatar. Bahrain found D8. In 2011, B3 was detected in Oman (also D8), Saudi Arabia and Bahrain (also D8). The B3 viruses were all within 3 or 4 nucleotides of each other but the D4 and D8 viruses were more diverse.

Ms Patcha Incomserb presented sequence data for SEAR. Four measles genotypes were circulating in the region between 2004 and 2010; D4, D5, D8 and D9. In 2011, only D9 (Thailand) and D8 (Nepal, Sri Lanka and Thailand) were found. Rubella genotype 1E circulated in Sri Lanka and Thailand from 2005 to 2009. Since 2009, 2B has been dominant. Genotype 2B was detected in Bangladesh, Nepal, Sri Lanka and Thailand.

Western Europe data was summarized by country.

Dr Sabine Santibanez presented an update on genotypes in Germany. D4 was the predominant genotype. Many of the importations into Germany were from France. Other genotypes detected were B3, D8, D9, G3 and HI, with most importations linked to European sources. The outbreak in Bulgaria has been terminated but there has been a resurgence of measles in Scandinavian countries. An absence of measles in Baltic States and several CEE countries was noted.

Dr Kevin Brown reported that D4 was the predominant genotype in England and Wales and a few D8 and some G3 genotypes. All genotype A cases had received vaccine within a month of testing. Importations were linked to 12 countries with the majority, D4 viruses, from France. Genotype G3 was identified in 6 countries at the same time and the sequence was found to be 100% identical.

Dr Judith Huebschen presented measles in the countries supported by the Luxembourg RRL. Most countries had outbreaks or sporadic cases caused by different variants of genotype D4. Outbreaks were reported from Spain (B3 and D4), Belgium (D4), Serbia (D4), Macedonia (D4), Turkey (D9) and France (D4) and especially from France viruses were exported to numerous other countries. Sporadic cases of D4 were found in Cyprus, Greece, Luxembourg, Netherlands, Portugal and Turkey. In
addition, genotypes A-vac (Spain), B3 (Serbia and Turkey), D8 and D9 (Spain), G3 (Spain and France) and H1 (Spain) were reported, with several of the cases being linked to importation.

**Dr Youngmee Jee** presented genotype data for the Western Pacific Region. In 2010 the genotypes found in the region were H1, D4, D8, D9, G3 and B3. D9 is endemic in the Philippines and Cambodia, H1 in China, Viet Nam and Lao PDR. G3 was found mainly in Malaysia and Singapore. Genotypes found in Japan were H1, D4, D5, D8, D9 and G3. Australia identified imported measles from multiple different countries.

Rubella genotype information is available from increasingly more countries in the region with genotypes 1E, 2B and 1j predominating.

The data for China was presented by **Dr Yan Zhang.** From January to July 2011 there were 9258 cases of measles, which is a 73% decrease from 2010. The age breakdown for measles cases in 2011 showed more than 20% in age group of <8 months and 30% in age group of > 15 years, highlighting age groups that need to be targeted for immunization.

H1 continues to be the predominant genotype with sporadic cases of d11 found in Yunnan province, genetically closely related to the outbreak strain from 2009. An outbreak of measles (H1) was detected in Hotan prefecture, Xinjiang Province, where the recent wild type polio cases have been found. Less diversity of H1 viruses appear to be circulating in 2010-2011 after the province-specific and nation-wide synchronized measles supplementary immunization activity in late 2010, in which more than 100 million children were vaccinated.

In AMR, endemic measles circulation has been halted and all sporadic cases are determined to be import related, although sometimes small outbreaks are linked to these cases. **Dr Paul Rota** provided an update. Repeated importation into the USA occurs from measles endemic regions, predominantly Europe and from a large outbreak in Canada, which started from an importation from France. In 2010 there were 4 outbreaks and 12 in 2011. The predominant genotype has been D4. Genotypes D8, B3, H1 and G3 have also been found in the US in 2011.

In the elimination phase it has been found that molecular studies alone cannot differentiate between continuous circulation and importation.

Molecular surveillance of measles in the Russian Federation and CIS region was presented by **Dr. Sergey Shulga.** In 2010 in Russia, 129 cases were confirmed. Eleven regions reported measles but 90% of cases were confined to just 3 regions. More then 70% of cases were linked to local outbreaks in two regions in the Asian part of Russia and resulted from measles H1 genotype importation from China. In Moscow, D4 was the predominant genotype but D8 was also circulating. A new variant of D4 was found in Tyumen which was closely related to a strain isolated at the same time in Uzbekistan.

In 2011, many cases and clusters of cases in Russia showed evidence of prolonged transmission of D4 genotype after importation from two main sources: Western European countries (D4 “Enfield” cluster) and Uzbekistan. The latter strains represented a new “cluster” of D4 genotype that had not been found in the CIS region before. However, closely related strains were shown earlier in Pakistan and Iran from were they were probably imported into Uzbekistan.

There were more than 400 cases in Uzbekistan during 2010-2011 which were genetically the same strains isolated from cases and outbreaks in neighbouring Kazakhstan, Kyrgyzstan and Russia, most epidemiologically linked to Uzbekistan. This lead to questions whether a resurgence of measles was occurring.

It appeared that measles in Russia from 2010 to 2011 was caused by repeated importations however these resulted in no or limited spread. A lack of collaboration between countries was identified as an ongoing challenge in the region.

**Dr Hein Boot** from RIVM, Netherlands, discussed the use of Luminex and protein arrays for IgG and IgM detection.

The main objective was to standardize IgG tests and provide rapid diagnosis. The Luminex technology uses antigen coupled to colour coded microspheres with up to 100 different antigens can be detected.
The test is specific and sensitive. Sample and antigen saving is possible and the stability is more than one year. Up to 500 samples a day can be tested. Future plans include testing for IgG, IgM and IgA, isotyping (IgG 1-4) and avidity. Microarray technology allows small volumes to be tested and multiple antigens in the one test. This allows differential diagnosis to be performed such as rash/fever investigation (measles, rubella and parvovirus). Serum, oral fluid, dried blood spots and B cell supernates can be used in this assay. Reference activities include independent testing, avidity and reagent preparation (beads and slides). Training courses and instruction of production of beads and slides is a possibility and collaboration with WHO programmes is considered a priority for RIVM.

**Dr Richard Meyers** gave an update on the MeaNS database and the problems with measles sequence analysis such as lineage expansion and classifying subtypes. The local diversity of the D4 measles genotype currently circulating in Europe is 8%. The genotype naming may not be descriptive enough but subgenotyping is likely to be unworkable. At present there are 2804 D4 sequences in MeaNS with the average change 8.83% and the maximum change 37%.

**Dr Alberto Severini** gave an update on the Canadian outbreak. The outbreak in Quebec was caused by a number of D4 genotype importations from France. To date, there were 739 cases, 101 were sequenced (N 450nt) and all sequences were identical. The outbreak in British Columbia was of unknown source and resulted in 9 cases, six were sequenced (N 450nt) and there was one nucleotide difference noted. An Ontario cluster was due to an importation from France, and resulted in 4 cases, all of which were sequenced.

**Dr Paul Rota** discussed the whole genome sequencing of 10 assorted viruses by J. Craig Venter Institute and the significance of the nomenclature. Some of the full genome viruses had an extra 6 nt in the non-coding region. There is a need to update the nomenclature document and also perhaps update the reference strains. A protocol to define a cluster needs to be made and the utility of expanding sequencing windows should be discussed.

**Dr Katsuhiro Komase** provided an update of genotypes circulating in Japan. From 2006 to 2008, D5 was predominant. From 2010-2011, D9, H1, D4, D8 and G3 have been detected either in sporadic cases or small clusters of cases and many cases had travel history to countries with measles outbreaks, indicating repeated importation.

**Dr Claude Muller** gave an overview of sequencing the N, P and H genes of measles viruses collected during outbreaks in Greece and Macedonia. From the D4 outbreak in Greece in 2010, 25 strains collected during 28 weeks were sequenced and 16 of them were identical in the N450 and nine further strains differed by 1 to 2 nts from the main variant. The maximum difference was 3 nts. From Macedonia, 18 strains were collected during a 10 week period and all of them were identical in the N450. In addition, D4 (8 sequences covering 14 weeks) and D6 strains (20 sequences spanning 20 weeks) collected during an outbreak in Greece in 2006 were investigated. In the N450, the D4 strains were identical and 3 variants of D6 were found. Using the combined NPH sequence data, there were 11 and 5 different variants among the identical N450 sequences from the 2010 outbreaks in Greece and Macedonia. The D4 cluster from the Greek outbreak in 2006 showed 2 sequence variants and the D6 cluster, which already showed 3 variants in the N450, resulted in 13 NPH variants. Thus, the NPH data provided a higher resolution for identifying possible separate importation events.

**Dr Joe Icenogle** discussed rubella sequencing. Sixteen virus isolates were selected for whole genome sequencing. The goal was to expand the number of rubella virus genomes sequences with an emphasis on recent isolates from currently circulating genotypes. Twenty-six percent of the nucleotides and 11% of the amino acid positions have at least one genome with a variant nucleotide or amino acid. Among the regions/domains, the HVR has the greatest variability (51%) while the MT has the least (12%). Genotypic specific deletions were observed in the intergenic region of all the 2B and 1G, 1h and 1i viruses sequenced.
Roundtable discussion
It was agreed that the original reference sequences were still robust but the old rules of % variation may no longer hold up. There was a need for a WER publication to describe naming structure for genotypes and variants. In the meantime the LabNet needs to find a way to tag strains in the MeaNS database for easy searches and analysis.

Dr Joe Icenogle gave an update on rubella genotypes. There are 13 genotypes, 2 clades. The structural protein coding region has 3192 nts.
From 2005 to 2010 genotypes 1E, 1G and 2B were widely distributed. 1a, 1B, 1C, 1h and 2C were geographically restricted.
The importations of rubella virus into the US could not be traced to their source. Joe suggested that we need a method to subdivide the genotypes in order to better track the viruses. He also suggested that 1h and 1a should be formally upgraded to accepted genotypes.
He listed a number of recommendations:
Determination of whole genome sequences of all reference viruses.
Establish accepted methods of subdividing the widely distributed genotypes.
Continue to establish a rubella sequence database.
Establish a simple system for communicating nomenclature changes.
Use of at least the standard 739 window is a necessity.

Dr Kevin Brown discussed the MeaNS database. The database stores measles sequences, genotypes. Comparison/phylogeny with viruses in other countries can be made. Formation of a steering committee to advise on the way forward has been set up. A different access level for different users is available. Phylogenic trees are becoming increasingly complex. The ability to download public sequences to analyse locally is also a feature.
The recommendation that data be submitted in a timely fashion was made.

David Featherstone gave an overview of the WHO database. 2480 new measles viruses have been submitted in the last 12 months. Forty-nine countries submitted data in 2011 with two countries submitting for the first time.
Three hundred and sixty-six new rubella viruses were submitted.
The challenges for the future are to fill the gaps in reporting for both measles and rubella. He reminded participants that there is an accreditation requirement to submit timely sequence data as the programme need timely results to help identify whether cases or outbreaks are due to endemic or imported strains.

Session 4: Enhancing Surveillance for Measles and Rubella

Dr David Brown discussed the issues to be considered in setting up serosurveillance. What needs to be addressed: Vaccine coverage, disease incidence, serological survey, vaccine effectiveness?
Seroepidemiology can monitor the performance of vaccine programmes or determine population immunity. There are two approaches that can be made.
1. Collection of serum banks using residual samples collected from laboratories.
2. Cluster population sampling
Standardization of laboratory methods for serosurveys needs to be addressed and the correct sample size needs to be calculated.
Collection of residual samples is cheap and convenient but how representative is it of the general population and the vaccine history is not known. The cluster population sample is more expensive and can be biased due to refusals. However it can be used to collect data such as vaccine history and oversampling of groups of interest can de done.
Is there a need for a guidance document was the question for discussion.

Oral fluid Surveillance in Africa was presented by Dr Charles Byahamazima. The main objective of the study was to determine the feasibility of using oral fluid in the filed to enhance measles and rubella
surveillance. The sample was found to be easy to collect and there is no additional shipping cost. Comparison of results from serum and OF collected from the same case showed high concordance. However the instructions on the device were found to be confusing and the supply of the devices was hard to maintain. Back-up staff and a ready supply of kits are necessary. Feedback meetings are also necessary to identify and address process problems. Determining the adequacy of the sample is a challenge and field training and support is necessary.

Dr Nalini Ramamurti described the pilot use of oral fluid samples for the detection of IgM and virus detection. India, Nepal and Sri Lanka were involved in the study. Results from India and Sri Lanka were presented with 200 serum and oral fluids from suspected cases collected. The OF samples were shipped at ambient temperature and the serum shipped under cold chain. For measles, the sensitivity of OF when compared to serum sample was 95-97% and specificity 78-83%. For rubella, the sensitivity was 72-83% and the specificity 98%. Training for adequate collection of OF was shown to be critical, however the OF was found to be a useful non-invasive substitute for serum. The virus detection component of the evaluation had not been completed at the time of the presentation.

Session 5: New Procedures and Technologies: Validation and Implementation.

Dr Claude Muller summarized the measles/rubella research group meeting held in Atlanta in May 2011. The aim of the meeting was to develop a research agenda for the next 5 years addressing some of the technical challenges in achieving measles eradication. There was five workgroups identified:
- Measles epidemiology
- Vaccine development and effectiveness, alternative delivery methods and laboratory methods.
- Immunization strategies
- Mathematical modelling and economic analyses
- Rubella.
Feedback on the document by all participants is strongly encouraged.

Dr Paul Rota reported on setting up alternative methods for measuring neutralizing antibodies to measles. To determine levels of measles protective antibody there is need to correlate antibody measurements with mIU/ml as measured by a standardized PRNT. However the PRNT is not easily undertaken and large scale studies require considerable resources and time, and finding an alternative assay is a priority. CDC has developed a microneutralisation assay using Edtag-gluc based on a 96 well format and read in a fluorescent reader. The assay takes a total of 3 days. The assay is planned to be validated with the PRNT assay.

Biomarkers of CRS in older children in Brazil was presented by Dr Joe Icenogle. Currently IgM detection and virus isolation is limited to CRS diagnosis in children <1 year. The aim of this study was to find specific markers that could aid in the diagnosis of CRS in children 6-12 years of age. A reducing Western Blot to determine E1, E2 and C, specific IgG titres and avidity were the tests that were trialled. The antibody to C appeared to be the most significant marker.

Dr David Brown gave an update on the point of contact (POC) test that has been developed at HPA. The test gave a sensitivity of 98.2% and a specificity of 90% when tested on Zimbabwe serum and Oral fluid paired samples. A company has been commissioned to make up a batch of these POC assays which will then be re-validated and if successful will be assessed under field conditions.
**Session 6: Quality Assurance and standardization**

A summary of the measles/rubella IgM proficiency results for panel 01005, distributed 2010/2011, was given by **Jennie Leydon**. Two-hundred and 223 laboratories participated in the panel testing. All laboratories scored >90%, therefore all passing the proficiency test, however a number of laboratories had results that differed from the optical densities that the majority of laboratories reported. An investigation into the testing procedure of these laboratories should be made. Sixty-three percent of laboratories returned their results to VIDRL within 14 days and 92% tested the panel within 14 days of receiving it.

**Dr Paul Rota** discussed implementing a molecular “practice panel” programme. The panels are to be sent out on FTA cards to ensure that no infectious material will be shipped. It was proposed to distribute the panels globally through the RRLs. The pilot programme would initially just be sent to the RRLs and a SOP for the programme will be developed before it commences.

**Dr Joe Icenogle** reported on kits that CDC has developed for rubella detection. Distribution of standard reagents and practice samples on FTA paper is proposed. There are three kits available:

- A diagnostic PCR kit
- A real-time PCR kit
- A genotyping kit.

**Session 7: Data Management**

**Ms Marta Gacic-Dobo** presented a review of HQ monthly reporting process. The main problem was that the case data and specimen data did not match up.

From the data collected, analysis was made of:

- Number of reported cases by WHO region.
- Measles case distribution by month and WHO region.
- Reporting of discarded cases.
- Measles incidence rate.
- Number of cases with onset data.
- Global distribution of genotypes.

**Dr Mick Mulders** discussed the CISID, a reporting system on infectious diseases in Europe that began in 1998. Data is collected for all 53 member states; it is a web-based tool for surveillance of VPD and monitoring immunization data. There are public users and CISID users, different users are assigned different rights which determine the amount of data they are able to capture. The system allows for linkages of data which relies on the EPID number assigned. CISID captures, presents and analyses data and subsequently disseminates information to relevant individuals and/or organizations.

EURO is also developing a laboratory data management system for measles and rubella based on the experience it has gained with the polio LDMS. It is a web-based system to upload laboratory data to a central server, linking epidemiological and laboratory data. It also offers a tool to assess the performance of each individual laboratory connected to this system.

A round-table discussion to assess views on weekly reporting to WHO gave varying results for each region:

- **AFR** - Most laboratories are reporting on a weekly basis, without too many difficulties
- **SEAR** - Most laboratories are reporting on a monthly basis. India is reporting weekly
- **WPR** - Weekly reporting would not likely be feasible in the near future
  - China collects data weekly but does not report to WHO. Japan does not report to WHO.
- **EMR** - Will discuss reporting at their next regional LabNet meeting in October.
- **AMR** - Weekly case based reporting not sample reporting is currently working well.
Session 8: Challenges of meeting Elimination Criteria for measles and rubella

Mr Gibson Woo Kei Sheng gave Hong Kong’s strategy for testing and reporting positive measles cases. The methods in use are Microimmune measles IgM EIA with Siemens IgM EIA as a confirmatory assay. Complement fixation assay is available but not used routinely. Measles virus isolation is performed as well as RT-PCR and sequencing.

Preliminary serological testing data showed that optical densities in the EIAs were higher for those infected with wild type virus than those vaccinated. Sensitivity of IgM detection in serum samples collected >4 days after rash onset was 100%. The Microimmune EIA IgM capture assay appeared to be more sensitive than the indirect Siemens EIA IgM assay. The testing algorithm in use is screening serum of patients presented with rash and fever by the Microimmune assay and if positive or equivocal, retesting with the Siemens assay will be performed. If the Siemens assay is negative the result reported is negative. A remark will be sent, stating that samples collected within the first 4 days of onset may be negative and a second sample should be collected if clinically suggestive. A nested RT-PCR will be performed on IgM positive serum samples and the highest positive rate (about 80%) was observed when collected within 7 days of rash onset. For non-serum samples, positive rate of RT-PCR was almost 100%, when collected within 16 days of rash onset.

Dr Katsuhiro Komase reported on false positive IgM results that were detected in Japan. Samples that were measles IgM positive by the Denka Seiken kit and PCR negative were found to be parvovirus IgM positive, PCR positive. Another set of samples measles IgM positive by the Denka Seiken kit and PCR negative were HHV6 or HHV7 PCR positive.

On evaluation the Denka Seiken kit was more sensitive but less specific than the Siemens kit. More thorough investigation and lab testing is necessary in the elimination phase to ensure an accurate diagnosis.

Dr Annette Mankertz analysed the German data to establish the correlation between IgM testing and PCR with respect to rash onset. Five hundred and fifty cases were analysed from 2005 to 2011. Samples were tested for measles IgM by Siemens from 2005 to 2010 (468) and by Microimmune in 2011(88). The correlation of IgM positive/PCR positive increased from 63% at day 0 rash onset to 100% at day 6 after rash onset. The conclusion was that the Siemens IgM and PCR testing are working as would be expected. A nested PCR was used.

Dr Sergey Shulga presented the experience of an outbreak of measles in Amur region in 2010, close to the China border. All serum samples were tested with several indirect and capture assays after an indication that sensitivity of the indirect was lower in previously vaccinated cases. It was found that the outbreak cases could be categorized into two groups. The first group consisted mostly of very young, previously unvaccinated individuals who were 100% positive by indirect and capture IgM assays; the second group were adolescents or adults, had been previously vaccinated and all positive by capture IgM but 33% were negative by indirect IgM assays. The second group also had high avidity and very high IgG ODs compared to the young, non vaccinated group. Clinically, the second group had slightly milder signs and symptoms. The investigation is being written up for publication by the Gabrichevsky Institute.

Avidity testing as an aid for diagnosing recent rubella infections was presented by Dr Joe Icenogle. When the incidence of rubella infection and CRS is low, the positive predictive value of specific IgM tests for confirming a recent infection is also low. Supplemental tests such as testing for the presence or absence of IgG antibody and IgG avidity help to identify a recent infection. The diagnostic sensitivity of the CDC assay was evaluated on a group of 25 samples with primary infection, ninety-six percent had an avidity <30%. The specificity was evaluated on 40 samples with a past infection and one hundred percent had an avidity value of > 40%. If a serum is collected less than 3 months after onset of rash, avidity should be low if it is a true rubella infection, however clinical and epidemiological data must also be taken into account. EuroImmun and Radim assays were found to be statistically better than other commercially available assays. The ranges of significant avidity vary by kit as does the kit's definition of a recent infection.
**Dr Marilda Siqueira** presented PAHO laboratory guidelines for measles and rubella case classification. These guidelines discuss issues related to quality control and case classification and laboratory testing for sporadic cases. In this latter, 5 situations are discussed. Using examples from Brazil, she stated that Brazil reported their last measles case in 2000 and last rubella case in 2008. In the elimination phase of measles and rubella, follow-up serology and case investigation is essential. She presented a number of cases where accurate follow-up was necessary for diagnosis and detecting the source of infection. Collection of an NPA and second serum samples after serology with IgM positive results were recommended for case confirmation.

**Dr Paul Rota** explained the use of RT-PCR and other assays for confirming measles. RT-PCR can help to confirm a case when the serology is inconclusive, however negative PCR result does not rule out a case of measles. The challenges for case classification and diagnosis in countries in or close to elimination include cases of primary and secondary vaccine failure, vaccine reactions that can be confused with disease and the collection of appropriate samples for virological surveillance. The use of further assays other than IgM is needed to classify a case and these may include IgG assays, avidity testing and PCR. Analysis of these assay results along with clinical symptoms and time of rash onset enables us to classify a case of measles, vaccine reaction or vaccine failure.