Report on the
8th WHO Global Measles Rubella Laboratory Network Meeting
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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 679 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 2010, the meeting discussed in 7 sessions the progress with the network, development of new technologies, progress with implementing alternative sampling methods, measles and rubella molecular epidemiology, strengthening quality assurance and standardization.

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.

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. WHO has learned from the legacy of smallpox, where surveillance played a key role in the certification process of smallpox eradication, differential diagnosis with monkey pox and other viruses. 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 as well as discuss operational aspects of sample transportation.

Discussions at a recent consultation held in July 2010 in Washington DC on the feasibility of measles eradication led to the conclusion that with the current scientific evidence available, global eradication of measles is achievable and highly cost effective. A target date of 2020 could be feasible if SEARO establishes an elimination target in the near future. The decision to adopt an eradication goal and setting a target date needs to be reviewed by SAGE and subsequently the Executive Board before it can be discussed by the World Health Assembly. However, the primary focus is for achieving the milestones for 2015: to reduce global mortality due to measles by 95% and achieving a measles
incidence of less than 5 per million through vaccination coverage of at least 90% in each Member State and at least 80% in each district.

The global financial crisis has impacted on the funding of the WHO Measles Programme. The Measles Initiative has seen a decrease in funding from a peak of $160m in 2005 to $60m in 2009. This decrease will create a challenge to conduct high quality follow-up campaigns and there is a need to intensify communication and advocacy 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, including the African Region’s 2012 pre-elimination goal and the South-East Asian Region endorsing the 2015 global mortality reduction target. A target date for elimination has yet to be established in SEAR. During the 2010 World Health Assembly, support for global measles eradication was obtained but no target date was set, but 2015 targets were set as milestones towards eradication of measles. In addition, AMR and EUR have set elimination targets for rubella and CRS.

Global routine measles containing vaccine coverage has increased from 71% in 2000 to 82% in 2009, but has levelled off in 2009. Progress has been achieved mainly through campaign style vaccine delivery. Overall, there has been a decrease in measles incidence, although AFR is battling resurgence. Reduction in measles mortality reached 78% in 2008 compared to the year 2000. Five WHO Regions achieved the 90% mortality reduction goal, with SEAR having reached 46% reduction, although all countries in the region except India achieved the 90% goal. India plans to start rolling measles immunization campaigns in November 2010.

The funding gap poses a risk of resurgence, with the ongoing outbreak in Malawi an example of what can happen if population immunity is not maintained. The recent measles vaccination campaign in China in September 2010 was the largest measles campaign ever conducted and was conducted successfully. Definitions have been proposed for elimination, endemic case and endemic transmission as well as imported case, import-related case and re-establishment of endemic transmission. Indicators for surveillance have also been proposed: reporting rate, at least 2/100,000 discarded measles cases; laboratory confirmation, at least 80% of suspected cases with adequate specimens; viral detection, at least 80% of lab-confirmed outbreaks with adequate specimens for virus detection; adequate investigation, at least 80% of all suspected cases with timely investigation within 48 hours. It is planned for these definitions to be finalized and published in the WER in the near future. Progress towards elimination can only be monitored in the presence of high quality surveillance and as measles incidence is an imperfect measure, verification committees will be established and the process for forming these is under development.

Dr. Alya Dabbagh presented an update on the feasibility of measles eradication. In May 2008 the Executive board of WHA requested an assessment of the feasibility of global measles elimination. In 2009 the term global "elimination" was changed to global "eradication". In 2010, both the Executive Board and the WHA adopted the 2015 target, without specifying a date for global eradication. The feasibility of eradication has been assessed during a global technical consultation held in Washington DC, July 2010. Seven facets were evaluated: biological, programmatic, vaccine market analysis, impact on health systems, economic analysis, risk analysis the post-measles era, global context and political feasibility. The key recommendations were: measles can and should be eradicated; a target date for measles eradication should be established once SEAR has established an elimination target; global eradication by 2020 is feasible (given measurable progress towards 2015 mortality reduction targets); measles eradication activities should be used to accelerate rubella control and the prevention of CRS.

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 679 and 183 Member States are served by a proficient laboratory. Over 98% of laboratories passed the WHO proficiency testing programme, with most achieving a 100% score. The workload for the laboratories as assessed by the number of measles serum specimens tested dropped
from a peak of 250,000 in 2007 to approximately 160,000 in 2009, mostly due to the decrease in measles cases in China. As measles cases decline globally, approximately 80% of all samples are also tested for rubella IgM. Most labs met the criterion of 80% of timely reports within 7 days of receiving the sample.

A number of new procedures are in the process of being validated including an RT-PCR EQA programme for measles and rubella, standards for measles serosurvey, and rapid point of care assays. Recently validated procedures are being implemented in the LabNet including; real time RT-PCR for measles and rubella, new sequencing primers, and the roll out of alternative sampling techniques.

Member States are enhancing surveillance to meet the indicators for achieving elimination. This has implications for the LabNet as there is a greater requirement to determine whether measles strains are imported or indigenous. Also countries need to meet the minimum rate of sampling which will result in an estimated increase of at least 50,000 samples per annum for IgM testing. Oral fluid alternative sampling techniques are in the process of being evaluated in five African countries and India to enhanced surveillance of measles and rubella. Training and capacity building remains an important component of the LabNet especially as new techniques are implemented, increased emphasis on molecular testing and the need to train new staff. Case-based surveillance, including laboratory diagnosis, has been implemented in most (94%) Member States although timely reporting to WHO of measles samples tested (100,000) are considerably fewer than the actual number tested (>225,000). Significant progress has been made with developing the global sequence database for measles and rubella with more than 8000 measles and 600 rubella virus sequences currently in the databases. However, there are still gaps, especially for rubella virus surveillance.

The LabNet has made good progress in recent years and has proven critical for adding specificity to surveillance information as incidence declines. The VPD LabNet contributes to the strengthening of health systems through human resource development. The training programme has resulted in 151 staff trained over the past 12 months. National diagnostic services have been strengthened as a result of this capacity building. Although the LabNet continues to perform at high levels the need to meet elimination surveillance criteria, including enhancement of molecular surveillance, will require additional resources to be found. Another challenge is the need to improve integration of laboratory and surveillance data and ensure that accurate and timely country reporting occurs.

**Regional LabNet updates, progress and challenges**

**Dr Annick Dosseh** presented an update on the MR LabNet in AFR. In many Member States especially in the West and Central blocs of the region, measles lab activities have been combined with Yellow Fever. Training, monitoring lab performance and accreditation are the pillars of the LabNet. Five training workshops have been held in the past 12 months for measles and yellow fever, with a focus on standardized IgM testing, QAP, data management, biosafety, molecular techniques and oral fluid testing. Some inefficiency in terms of timeliness of reporting data to WHO have been noted. Most labs in the AFR network are performing adequately and all are accredited or provisionally accredited.

Genotype information on African strains is becoming of increasing importance and additional training has been provided for collecting appropriate samples (including oral fluid samples), measles virus isolation and identification.

Many challenges remain, however, including shortage of funds, adequate supplies of kits, equipment, high staff turn-over, competing activities of polio, YF, influenza and other priority diseases.

An update on the performance of the EMR LabNet was presented by **Dr Hinda Ahmed**. EMR has already achieved the 2010 Regional Elimination goals, but is facing challenges in terms of security and finding sufficient resources. The majority of measles cases are reported from GAVI-eligible countries, particularly in Member States with security issues (Iraq, Somalia, and Afghanistan). Despite these challenges, measles validation guidelines have been prepared.

Laboratory performance (based on performance indicators, PT testing, accreditation status, confirmatory testing) is adequate and has improved since last year. Laboratory staff from 12 countries...
underwent training, including molecular training, in two workshops conducted during 2009 and 2010. As sequencing capacity in the Region has improved through the training workshops, considerable information is now available on the measles and rubella genotype distribution in the region. For measles, D4 genotype and B3 continues to be the predominant viruses detected and rubella genotypes detected are 1E, 1G and 2B.

A study conducted in Iran and Syria showed good concordance between serum spotted on filter paper and the original serum specimen in terms of measles and rubella antibody testing and gave similar results when also tested at the RRL in Muscat, Oman. Despite Regional progress, challenges remain for the Measles Programme and for the LabNet in particular, including case-based surveillance, timeliness of investigation, collection of specimens, monitoring virological surveillance and high staff turn-over. Governments are being encouraged to incorporate support for National measles laboratories as part of their national surveillance programmes.

The European Region has made a renewed commitment to measles elimination and has revised the goal to 2015, as presented by Dr. Galina Lipskaya. Vaccination coverage was reported as lowest in the West-European countries. A large measles outbreak occurred in Bulgaria among the hard-to-reach Roma population. Despite the fact that a number of countries are in the elimination phase, some countries are facing re-establishment of endemic circulation. For rubella, the situation is better for the Western EUR countries. Most rubella cases occur in the East, as immunization efforts have been commenced more recently. Rubella genotype 2B caused large outbreaks in both governing entities of Bosnia and Herzegovina.

Despite the absence of a European LabNet coordinator, the LabNet has remained in relatively good shape and performing adequately, mostly due to the high commitment of the RRLs. Oral fluid samples are increasingly being used throughout the Region. Additional training will be needed to benefit more from this technique.

Challenges also remain in EUR. MR data quality needs to be improved by integrating laboratory and epidemiologic components of surveillance, but also to ensure data is received from sub-national laboratories and multiple clinical diagnostic laboratories in some countries in the region. Challenges also remain for achieving the CRS prevention goal by 2015. Laboratory activities have to be stepped up, but also CRS surveillance. Currently, there are a number of laboratories in the LabNet which receive insufficient support from their national governments to be sustainable without external support.

According to Dr Youngmee Jee, measles elimination is on track in the Western Pacific Region. However, outbreaks, occurred in Viet Nam, the Philippines, and New Zealand. Good progress has been made in Japan, and China, and overall 25 countries have eliminated measles. A Regional and National Verification Committees will be formed.

The Regional LabNet has been performing adequately and 10/13 national laboratories have been accredited as of September 2010. All national laboratories have established confirmatory testing with good concordance rates of 90-100% in the past 12 months and all labs passed the WHO PT test. Recent measles genotype data is available from most countries. While H1 and D9 strains are most frequently reported in the region, G3, D4, D5, D8 and B3 strains were also detected from sporadic cases, as well as from outbreak or imported cases. The 3rd Regional hands-on training was held in the Hong Kong China RRL in 2009 and a follow up training on molecular diagnosis will be organized in November 2010. The China LabNet has implemented the WHO Global EQA programme and most labs conduct virus isolation and MMR Q-PCR.

Challenges for WPR include reporting of case-based data from China and Japan, testing algorithms in some countries, insufficient government support for national labs, timely procurement of diagnostic kits, and collection and transportation of samples for further virological analysis.

The role of the MR LabNet in the American Region was discussed by Dr Marilda Sequeira. AMR has achieved measles elimination and rubella cases have not been seen for the past 19 months. AMR is now moving to the stage of documenting and verifying absence of measles, rubella and CRS. Several Member States have established national verification commissions. The AMR LabNet has an
important role to play as good surveillance is a critical component of the verification process. The role of the lab will be to ensure timely case confirmation and identification of the etiological agent, including sequence information. The lab will have to deal with many challenges, including detection of low levels of IgM antibodies, false positive and false negative results, decreasing positive predictive values in low incidence settings, differential diagnosis and virus detection. It will be critical that reconciliation of epidemiological and laboratory data occurs.

In terms of measles control, the South-East Asian Region is the most challenging, according to Dr. Nalini Ramamurty, who will be retiring from WHO next year. Vaccination coverage has not reached the desired levels, particularly in the two most populous countries of the Region, India and Indonesia. However good progress has been achieved in the other Member States in the region. To accommodate the demands of increased surveillance, the regional LabNet has been strengthened through capacity building workshops, with a focus on accurate serological testing and enhancing molecular surveillance. Measles and rubella genotyping data is now available for most countries in the region except Bangladesh and Bhutan, and Indonesia has not reported sequence data since 2006. The LabNet laboratories are performing well and all have been accredited. Challenges remain in terms of linking lab and epidemiological data, funding, implementing alternative sampling techniques for serology and sequencing, increased work load, especially when case based surveillance in India is implemented.

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

Dr. Charles Byabamazima presented the experience in AFR with measles surveillance now that the region has set a pre-elimination goal of 2012. Key indicators have been set and some countries are meeting these performance indicators. However, many countries are facing challenges to meet these indicators. Large outbreaks have been occurring throughout the region and though routine coverage has improved, it has levelled off since 2006. Most key measles surveillance performance indicators have improved since 2006. The use of epi-linking to laboratory confirmed cases should be made in the countries experiencing large outbreaks so as not to overwhelm labs with the testing of large number of samples. Finding sufficient funds for maintaining progress with the LabNet is another challenge.

Several reports were made on measles genotype distribution. Sheilagh Smit reported on the results of her activities in support of measles molecular surveillance in AFR. Genotype B2 was circulating in Namibia and Angola in 2009 and 2010. Occasional evidence of importations from other WHO-regions into South Africa was reported including D8 from India and D4 from Europe. Genotype B3 virus is circulating widely across Africa but distinct variants have been found. Most measles virus genetic information is collected from serum samples sent to NICD for confirmatory testing and the gaps in molecular surveillance are gradually being filled.

Dr Henda Triki showed recent molecular data from EMR, where molecular surveillance has been well established due to the efforts in building capacity in the region, especially with the support of CDC and the two RRLs, Oman and Tunisia. The predominant genotypes found were D4 and B3, and a new sub-cluster variant of B3 was found in Libya and Tunisia in 2009. Additional genotype data from EMR was presented by Dr. Suleiman Al-Busaidy, who showed predominance of D4, D8 and B3 strains in the Gulf and surrounding countries.

In SEAR, the genotype distribution is slightly different according to Patcha Incomserb. The predominant measles genotypes in recent years were again D4 and D8, although D5 and D9 have also been found frequently. Most sequences reported were found in Thailand, and only very few were received from India, and Indonesia. However, both India and Indonesia laboratories have sequencing capability and India reports sequence information regularly to WHO (D4 and D8). Genotype G2 was found in Indonesia and Thailand from 1998-2001 which has not been seen since except for an
importation into Australia in 2004 from an unknown source. Limited genotype information has been obtained for rubella, but 2B and 1E were the predominant genotypes found. Based on sequence information, tracing virus transmission pathways has proved to be difficult, primarily due to the incompleteness and insufficient representativeness of the sequence database. Countries in the region are urged to submit samples for genotype analysis to the LabNet and to include complete epidemiological information. WHO could facilitate. Other challenges dealt with ownership of sequence data, high workload and the need for computerization.

According to Dr. Aili Cui, there has been significant progress with measles elimination in China (25% reduction in cases in 2009 vs. 2008, and 32% in 2010 vs. 2009) due to the considerable efforts of the national measles programme. The China LabNet follows the same principles as the Global LabNet, including the emphasis on a strong QA programme which includes; confirmatory testing, global proficiency testing, annual training, and accreditation with on-site reviews completed by international experts. In 2009, all 31 provincial labs participated in the WHO Proficiency testing for the first time and passed with maximum score. Genotype surveillance has now also been well established and H1 has almost exclusively found for almost two decades. Despite the high proportion of H1 strains, the surveillance system was able to detect a small number of imported cases, and the LabNet confirmed D9 and a new genotype, d11. Rubella genotype information is available based on 261 sequences isolated between 1999 and 2010. In recent years, the predominant genotype is 1E.

In AMR, endemic measles circulation has been halted and all sporadic cases are determined to be import related though 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. Sporadic importation of measles also occurs in Latin America. Molecular epidemiology has its limitations: molecular studies can confirm independent sources of infection if different genotypes or clearly distinct lineages are detected, but molecular studies alone cannot differentiate between continuous circulation of virus and multiple introductions from the same source. Expanding the sequence “window” may be able to overcome these limitations.

Following the 2010 Winter Olympics in Vancouver, 84 measles cases occurred in Canada according to a report by Dr. Alberto Severini. Molecular analysis showed several distinct genotypes, most were H1, but also 2 distinct variants of D8 were found.

Another potential source of measles virus transmission was the 2010 Football World Cup held in South Africa which was in the middle of a large measles outbreak. Many UK fans (>21,000) travelled to South Africa and there was a risk of these fans being infected in South Africa or of UK fans carrying measles virus to South Africa. Five cases were detected in the UK post World Cup who indicated history of recent travel to South Africa. Only 2 cases were consistent with World Cup attendance and the others were apparently infected in South Africa prior to the World Cup. Other examples of opportunities for the risk of measles spread following mass gatherings was the visit of the Pope to the UK, with many “Irish Travellers” attending and the upcoming Olympic Games to be held in London in 2012. The importance of sharing sequence information, making identification of spread following global events more easy, is critical. Discussions were held to formalize the sequence cut-off to define or exclude an epidemiologic link. Identical or one nucleotide change over the N450 nt was considered to be a determinant of possible linkage.

Molecular surveillance of measles in the Russian Federation was presented by Dr. Sergey Shulga. Currently, the incidence in the Russian Federation is low (1/1,000,000), but sporadic cases with limited spread (mostly nosocomial in very young <1 year, and young adults 18-35 yrs, including medical staff) due to importations continue to arise. The two main routes of importation are most likely from Thailand (Genotype D9) and China (H1). D4/Enfield was also found in Kyrgyzstan and Russia. The RRL in Moscow reported difficulties in obtaining samples from cases and is not able to reach the genotyping performance indicator of 80% of all cases stipulated in their national decree. Currently only aggregated data is available on Rubella in the Russian Federation, but case-based surveillance will be implemented in 2011. Since 2007, the predominant genotype is 1h and has shown decreasing sequence diversity, possibly as a result of successful intervention efforts.
Drs. Kevin Brown, Sabine Santibanez and Judith Hüebschen provided an overview of the genotype distribution of measles and rubella in Europe. Measles genotyping is performed on most IgM positive samples sent to HPA for serological investigation. The predominant genotype variant in the UK 2007-08 was D4/Enfield and again in 2010. In 2009 and 2010 many minor variants (1 nt divergence from Enfield) were also found. In Germany, various limited outbreaks have been reported with D4 predominant, but also B3 and D8 found. Switzerland also found D4 and sporadic cases of B3 and D8. D4 was found also in Austria, Italy, Romania, and Croatia. In France, D4/Enfield variants circulated widely and outbreaks caused by genotype D4 strains were also reported from Greece and Spain. Rubella genotype 2B caused large outbreaks in both governing entities of Bosnia and Herzegovina.

Dr Santibanez stressed the need for enhancing molecular surveillance, as she demonstrated that the co-circulation of many distinct variants indicated repeated importation events, sometimes coinciding with short chains of transmission, rather than sustained transmission within the country. In other West-European countries D4 was the predominant genotype as well. France reported widespread D4 circulation of an Enfield-variant. Rubella genotype 2B was found in Bosnia-Herzegovina.

Japan was a signatory to the WPR measles elimination goal of 2012. According to Dr Katsuhiro Komase the challenge has just started in terms of closing the immunity gap and enhancing national surveillance, including molecular surveillance. In 2009 and 2010, measles incidence has dropped significantly, but this may be attributed to supplementary vaccination efforts and also the large nation-wide outbreak of 2007-08. Currently only limited sequence information is available (9 sequences in 2009 and 8 sequences until August 2010). After the D5 outbreak in 2007-08, some sporadic cases were import-related and they were caused by D9, D8 and H1 genotype strains of MV. It was reported that only 70% of cases have laboratory follow-up, but this number is increasing. Approximately 95% of the diagnostic tests are done in commercial labs which do not have the incentive to forward specimens to the national reference lab for further characterization. Another challenge was the decrease in PPV with lower incidence and evidence that the commonly used Denka Seiken kit could show false positive measles results with parvovirus B19 IgM antibodies.

Dr Wilina Lim presented data on the molecular surveillance of measles and rubella from the Hong Kong RRL. The predominant measles genotypes in the Western Pacific Region are H1 and D9. For Rubella genotypes these are 1E, 1j and 2B. Challenges are the lack of adequate clinical and epidemiologic information, the use of non validated reagents and kits, insufficient sample volume for confirmatory and further testing, inappropriate sample transportation conditions, and for rubella, the need to encourage samples other than serum specimens for molecular testing.

In China a new genotype d11 has been recognized. It was isolated during an outbreak on the border with Myanmar and may have been imported into China. However, no data is available on the genotype distribution in Myanmar as surveillance is not adequate in the far eastern region. Dr Aili Cui requested that the d11 genotype be formally adopted. A recent article on the virus is published in EID. A request is made to share preliminary genotyping data within the MR LabNet as soon as it becomes available as misleading information was released by the Russian press.

Prof Claude Muller proposes to increase the sequencing window to increase the resolving power of molecular epidemiology. He provides several examples to make the point that analysing the hypervariable carboxy-terminal N-gene only provides limited resolution and too little sequence variability to link outbreaks and sporadic cases. As measles virus has a relatively stable genome, only a longer interval will show some sequence divergence and can resolve putative epidemiological links based on the fixation of mutations.

Based on the vast sequence database of the ongoing D4 outbreak in the UK, Dr Kevin Brown agrees that genotyping based on 450 nt N gene interval alone is insufficient to analyse epidemiological linkage. What is currently being seen is a high sequence divergence in the D4 sequence pool, as the outbreak progresses. More and more variants evolve from the common D4/Enfield ancestor. He questions whether the 2% demarcation is still appropriate now that more sequences become available. Critical is the quality of sequences as a single nucleotide difference can already discard an
epidemiological link. He also confirms the importance of good epidemiological data and the timely sharing within the LabNet of sequences. Studies are needed on a large set of measles strains from within a given region and with detailed epidemiologic information to determine linkage based on sequence information and to confirm with the available epidemiologic data. In this way, the molecular information based on a predetermined and potentially large sequence window, is calibrated against the epidemiologic information. A higher resolving power is needed particularly in the end-game.

Currently D4/Enfield was one of the most abundantly circulating strains, also outside the UK. Dr Annette Mankertz reported the D4 story from the German perspective. A limited outbreak occurred in Hamburg 2009 involving 278 cases. The index case of this outbreak became infected in London. More importantly, however, the index case of the large Bulgarian outbreak imported the virus from Hamburg. The outbreak consisted of more than 24,000 cases and affected mostly the ethnic group of the Roma. The virus also spread to other parts in Europe from this outbreak. Again, good epidemiological data, linked with sequence data is critical to gain the most information about outbreaks.

Dr Joe Icenogle provided an updated overview on Rubella virus genotypes, including their geographical distribution. He proposes three provisional genotypes to be formalized: 1h, 1i, and 1j. It is desirable to have two reference viruses of which each reflects sequence diversity within a genotype, as much as possible. He also discussed the long epidemic cycle of rubella and the higher genetic variability as compared to measles virus, although not as much as poliovirus (1/15th). He demonstrated that rubella virus can be isolated by using the measles cell culture isolation protocol with the last step being a staining or RT-PCR step to confirm infection. Also, the CDC developed in-house kits for RT-PCR, genotyping, Q-PCR and staining, and these are now available to the network.

**Session 4: Enhancing Surveillance for Measles and Rubella**

A collaborative study was presented by Dr Josephine Bwogi on differential diagnosis of cases with measles-like rash/fever that were measles and rubella IgM negative. The study showed a high seroprevalence for parvovirus B19, as well as HHV6 and HHV7. The latter two pathogens were the most common cause of measles-like rash/fever besides measles and rubella.

Dr David Brown highlighted the role of the laboratory in determining the etiologic agent in cases with rash and fever. In 50% of the clinically suspect rubella cases another agent is found, particularly dengue, HHV6, parvovirus B19. Rubella is only found in 10% of these cases. The remaining other half of cases the etiologic agents remains unknown. It is important to inform surveillance focus and the rate of rash/fever may vary by country, particularly in arbovirus-endemic countries, where it may mask measles and rubella. Furthermore, a testing algorithm needs to be defined, as has been done in AMR, when reaching elimination. More studies are needed to understand the epidemiology of exanthemous diseases. Furthermore, differential diagnosis may be difficult to sustain financially.

To enhance surveillance in the AFR, a pilot study is being conducted in five countries to assess the usefulness of oral fluid. Dr Charles Byabamazima presented the results. Samples collection, transportation and laboratory analysis was in general perceived simple and easy to perform. However, collection devices are not available locally like blood collection syringes and some basic training was deemed necessary. It was mentioned that in the current Zimbabwe outbreak, OF samples were able to confirm measles cases in babies before they developed teeth (< 4 months) and in samples collected in the first 2-3 days after onset. RT-PCR testing on these specimens can facilitate the collection of molecular surveillance information. Virus may also be cultured if a cold chain is utilized and the extraction buffer does not include Tween 20. HPA reported that they no longer use Tween 20 in their OF extraction buffer with there has been no apparent loss in IgM or RNA detection sensitivity.
Session 5: New Procedures and Technologies: Validation and Implementation

At CDC, several new procedures have been developed to facilitate sample and virus transportation for analysis at the RRL or GSL. Dr. Paul Rota presented a method to dry measles or rubella virus isolates on filter paper (Whatman FTA elute micro card). These should be shipped as Category B infectious substances (UN 3373, triple packaging). Also PT panels and control RNA for RT-PCR can be shipped using this procedure. RNA stability is satisfactory for at least 1 month and shipment can be made at room temperature. Avidity testing and PCR can be used to confirm difficult cases, e.g., vaccine reaction, recently vaccinated, primary or secondary vaccine failure. Real-time RT-PCR can be used in low incidence settings as it is more sensitive and can help to confirm serologic results. Protocols and primer sequences (including improved and more sensitive primers for measles genotyping) have been made available for the LabNet. A new measles genotyping kit with a positive control is also available for the LabNet.

Dr Joe Icenogle presented new developments for real-time and conventional diagnostic RT-PCRs and a method for genotyping rubella virus using RNA extracted directly from a clinical sample without using a nested primer set. Based on sequences from a new genotype 2B variant, a new primer was developed to be used with the original reverse primer in a 3-primer setup. He also highlighted the importance of using a special RNA extraction kit for GC-rich RNA, as in rubella virus. As obtaining a rubella virus sequence directly from clinical material is difficult, a two-amplicon system was developed to increase the success rate by about 10-fold. Rubella copy numbers vary between <10 and >5,000 per 2.5 µl serum specimen.

According to Dr David Brown, HPA has been developing a measles Point of Care Test (PoCT). Many different types of PoCTs are currently commercially available. A mumps IgM Near-Patient test for oral fluid developed by HPA has good performance (sensitivity 80% and specificity 100%). Based on the same design, a measles NPT performed similarly with serum (sensitivity 91%, specificity 94%) and also showed promising performance with oral fluid (sensitivity 89%, specificity 90%). PoCT seem very promising for measles surveillance, but needs to be evaluated under field conditions and for molecular analysis. PoCTs have potential to make a significant contribution to measles surveillance through rapid confirmation of acute infection, IgG detection can determine population susceptibility and oral fluid PoCT samples can be used for RNA detection. However, it is unclear what the commercial potential for such a test will be.

The result of the WHO Measles/Rubella IgM proficiency testing on panel 00905 was presented by Jennie Leydon. Samples have now been filtered to avoid contamination. An additional 31 Chinese provincial labs had participated for the first time. The Russian Federation used kits produced by Vektor/Best and EKolab which had reasonable performance. Prolonged storage at 4°C showed samples number 16 and 17 had declining OD values with time. This had an impact on the labs that tested the panels several months after being shipped from VIDRL. Sample 16, which had an OD value close to the cut off, was found by most labs which received the panel late to be equivocal or negative. These labs were not penalized and were scored out of a possible 19 (rather than 20) if they did not get a positive result. The overall passing score of the network laboratories continued to be very high (99% with >90%). It was proposed that additional points will be deducted in the future for: incorrect test validity criteria, use of expired kits, inaccuracy of reported data, transcription errors and incorrect interpretation of results.

To ensure standardization, control and analysis of serosurveys across the LabNet, David Featherstone suggested following points to be checked: appropriate sample size, and type of assay (PRNT). Within a few months, WHO will provide a protocol on conducting serosurveys. Emily Simons presented the Measles Strategic Planning tool to evaluate national immunization data. It also provides a means to evaluate population immunity and thus obviates the need to conduct serosurveys. The tool facilitates simultaneous evaluation of surveillance data and coverage data and quickly provides in-depth perspective on population immunity including estimation of age groups at risk of infection and effectiveness of immunization strategies.
As the measles control programme is placing increasing reliance on molecular techniques for determining progress with countries elimination activities, there is a growing need for a quality control programme for these molecular methods. Dr Paul Rota went through the precautionary measures a laboratory should take to conduct molecular testing and genotyping. He also presented a new version of the CDC measles genotyping kit. FTA cards are made available for QC purpose and as practice material. A PCR and genotyping proficiency programme needs to be developed. Internal (spiked) controls still need to be developed further.

Dr Joe Icenogle also presented tools for quality assurance of rubella testing. He has developed a cell line expressing viral RNA which can be used for developing molecular standards. The cell line is a transfected BHK cells with RV structural protein coding region with an insertion of 30 nts in the E1 gene and labelled SP-E1cMyc. The cell line is stable and does not require Geneticin. The type of RNA produced by this cell line may be more stable than the commonly used synthetic RNA transcribed from a cDNA clone, as cellular RNA is present. It can also be used as an extraction control and the copy number is constant making it useful for (semi-)quantification and can be used as a positive control for the ICA. An added advantage of this cell line is that it is non-infectious.

On behalf of EUVAC.net, HPA conducted under the leadership of Dr Kevin Brown a survey among 15 EU Member States on the extent of PCR testing for measles and rubella. The majority of laboratories do use PCR for M&R and most of them had SOPs. When presenting the results at a recent EUVAC meeting, there was general concern about obtaining material for validation of assays, especially in countries with few measles cases. There was also enthusiasm for production of a run control and for proficiency panels for molecular detection and genotyping of measles and for similar reagents for rubella. HPA has received funding to move to the next phase. With an increasing use of oral fluid sampling in the programme, the need for an OF specific EQA is becoming critical.

Dr Kevin Brown also presented the recent progress made with MeaNS (www.who-measles.org), the Measles sequencing database hosted at HPA. It now includes over 5000 measles sequences and also has increased functionality, including a reporting tool to EURO and WHO, and a GenBank upload tool. It is planned for trace files to be uploaded allowing sequence quality to be checked. A mapping tool will also be added. The sequencing lab can now also enter details on a specific sample submitted by the NL. A steering committee will be assigned to review policies for submission, download and use of sequence data.

In parallel, the WHO maintains a Measles and Rubella genotype database which now contains 8163 Measles sequences and 660 Rubella sequences (http://Workspace.who.int/sites/genotype/Genotype). David Featherstone reported a marked increase in submission of sequences and timeliness of reporting, with 1480 new measles viruses submitted and 17 new countries reporting in the past 12 months. Despite this, genotype information is still lacking for some countries reporting measles cases. New summary maps are posted on the SharePoint site and an automated mapping tool is being developed by Paul Chenoweth, similar to that developed for polio. Case-based data should be provided to the sequencing laboratory to allow all variables to be added to the database. Changes on-line to previous submissions, should be notified to the curator. Submitting measles viruses to MeaNS, allows all reporting criteria to be met and also assists with characterization and QC of the virus sequence. At a minimum, measles viruses must be submitted to the WHO database and preferably to MeaNS and GenBank as well. Rubella virus genotype information should still be submitted to the WHO database and preferably also to GenBank.

Lastly, Dr Paul Rota presented a sequence annotation tool developed at his laboratory. It functions as a stand-alone application and can perform BLAST searches, calculate multiple alignments and create phylogenetic trees. It also can export data to other programs. A compiled version can be made available for use in the LabNet. In the future, a link with MeaNS and the WHO database will be explored as an option.
**General discussion**

1. Additional space is requested to insert comments in WHO sequence database.
2. A steering committee for sequencing issues needs to be appointed.
3. MeaNS should become an annotated database and provide additional information that can not be provided in GenBank.
4. Rubella virus reference sequences should be posted on WHO sequence database.
5. The transition to weekly reporting is considered challenging due to the current workload and the communication challenges. Even monthly reporting remains challenging in some regions though the region of the Americas reports weekly. As more countries move closer to achieving elimination, weekly reporting needs to be implemented to allow a more immediate response to outbreaks.
   a. It is possible to report weekly, but reasoning needs to be clear, resources to be identified and more feasible when approaching elimination.
   b. Field epi information is perceived as a bottle neck. Therefore, regular meetings between lab and epi becomes critical, especially to reconcile data from the laboratory and field.
6. Inclusion of non-laboratory network data, particularly from private commercial laboratories is particularly challenging in Japan (and other countries). Commercial laboratories do not receive any incentive to report and no mechanisms exist to encourage them to report. This is a problem also perceived in other Member States with private health care providers.

**Recommendations: 8th Global Measles Rubella Laboratory Network Meeting, September 2010.**

1. **Funding**
   LabNet should endeavour to find additional resources and new partners. However, given the current global financial crisis, countries are encouraged to include laboratory support in their surveillance budgets. Additional funds will be necessary to support the training programmes required to maintain the current high level of LabNet performance, to support the introduction of new techniques and to hire new staff. **Action:** Measles Programme, Measles Initiative, LabNet. **Timeline:** Ongoing

2. **Data reporting**
   There are a small number of countries which have yet to establish a regular procedure for reporting laboratory-based data to WHO and in some countries, laboratory based data are not directly linked to surveillance data. Members of the LabNet are encouraged to work with their national surveillance programmes to reconcile laboratory and surveillance data and to ensure this information is sent to WHO according to agreed upon reporting requirements. **Action:** Regional programme focal points, LabNet Regional Laboratory Coordinators. **Timeline:** Ongoing

3. **Indicators for elimination**
   LabNet laboratories in the regions targeting measles and/or rubella elimination goals will be required to meet elimination criteria surveillance indicators, including a requirement that >80% of laboratory-confirmed measles outbreaks have adequate samples for virus characterization tested in an accredited laboratory. To determine the additional resources needs for the LabNet it is recommended that reference and sequencing laboratories estimate their current ability to reach this indicator and determine the additional resource needs to fully meet it. **Action:** LabNet Sequencing Labs **Timeline:** First quarter 2011
4. **Proficiency testing programme**
The LabNet continues to perform to a very high level in the serological PT programme. It is recommended that the scoring system be revised to be more compatible with testing routine samples and should encompass:

- Test results
- Completeness of providing all data used to determine test validity, including; kit lot numbers, cut-off values, positive and negative controls
- Use of valid kits
- Monitoring of transcription errors
- Correct interpretation of results

A new scoring system will be established to include the above parameters and will be introduced for the 2011 PT panel evaluation. **Action:** WHO HQ, VIDRL and LabNet

**Timeline:** First quarter 2011

5. **New techniques**
The new sequencing primers and positive controls for measles and rubella developed by CDC will be introduced to the LabNet with the appropriate protocols and will be provided at training workshops and to labs on request. **Action:** CDC **Timeline:** Ongoing

6. The procedural section of the Laboratory manual will be re-evaluated and updated where necessary. The key procedures identified for revision or development are:

- trouble shooting guide for ensuring optimal cell sensitivity,
- performing a serosurvey,
- use of new RT-PCR and sequencing primers
- appropriate use of real time RT-PCR
- QA for molecular techniques
- QC for oral fluid

The revised techniques/protocols will be posted on the WHO LabNet website and there will be consideration for publishing the revised manual electronically.

**Action:** WHO, GSLs, LabNet **Timeline:** First quarter 2011

7. **Point of care rapid measles assay**
The newly developed measles rapid point of care (POC) shows promising sensitivity and specificity when compared with detection of IgM in serum by EIA. It is recommended that the POC is further validated using samples collected under routine field conditions. **Action:** HPA, WHO HQ and AFRO **Timeline:** End 2010

8. **Sequence sharing**
The sequence information generated by the LabNet over the past 4 years has been considerable and has proven invaluable for planning programmatic action. This data will become increasingly important for monitoring national and/or regional progress in attaining measles elimination. Timeliness of reporting has improved but there is evidence that some labs are waiting for publication before reporting information to WHO and/or the databases. Labs are reminded that sequence information is most useful when it is shared in a timely manner and of the LabNet requirements for reporting sequence data within 2 months of sample collection but preferably on a real time basis. **Action:** LabNet **Timeline:** Ongoing

9. **Sequence Databases**
The WHO and MeaNS sequence databases are simple to use and have proven invaluable for monitoring and sharing sequence data. Timely submission of sequences to MeaNS allows all reporting criteria to be met in one action as these data are automatically submitted to the WHO database and optionally to GenBank. MeaNS also assists with characterization and QC of the
virus sequence. However, at a minimum, measles virus genotypes are to be submitted to the WHO database, either through MeaNS or directly. For all rubella virus genetic information, the WHO database should be used with sequence data preferably also submitted to GenBank. 

**Action LabNet Timeline:** On-going

### 10. Data requirements

The minimum epidemiological data required for submission to the WHO databases are: WHO name, place and date of virus sample collection. Regional coordinators and country offices are to help collect any missing data. Changes on-line to previous submissions to the WHO database should also be directly notified to the curator to ensure they are reflected in the "master" database. 

**Action LabNet Timeline:** On-going

### 11. Rubella genotypes

The SP sequences available from proposed reference viruses for provisional genotypes 1h, 1i, and 1j are: 1h: Minsk.BLR/28.05 AM258945; 1h: Novokuznetsk.RUS/04 EF421977; 1i: London.GBR/86 completed at CDC-USA; 1i: Milan.ITA/46.92 completed at CDC-USA; 1j: Kagoshima.JPN/22.04 AB285129; 1j: Miyazaki.JPN/10.01 AB285130. WHO should seek to have these viruses deposited in the WHO rubella virus strain banks to complete the process of making 1h, 1i, and 1j recognized genotypes. 

**Action LabNet, Timeline:** Agreement by end 2010

### 12. Measles genotypes

Provisional genotype d11 should be recognized as a new measles genotype, D11, with the sequences of the reference strain, MVv/Menglian.Yunnan.CHN/47.09/1, provided by China CDC. Virus should be send to the WHO Strain Banks. 

**Action China CDC, Timeline:** October 2010

Recognition should be given after laboratories have been able to analyse the reference sequences and report to WHO. 

**Action LabNet Timeline:** Agreement by end 2010

The genotype B3 sequences from Libya, Tunisia and Sudan should be considered as a third cluster in genotype B3. Reference sequences to be provided by Institut Pasteur de Tunis. Virus from this cluster should be send to the WHO Strain Banks. 

**Action RRL Tunis, Timeline:** (October 2010)

Recognition should be given after laboratories have been able to analyse the reference sequences and report to WHO. 

**Action LabNet Timeline:** Agreement by end 2010

LabNet laboratories should consider uniform naming convention for subgroups within genotypes especially those that have global circulation patterns. The recent changes in the list of recognized rubella and measles genotypes should be published in the WER. A draft will be circulated to all GSLs, RRLs, and Regional Lab Coordinators. 

**Action: CDC, LabNet, Timeline:** Draft by first quarter 2011

### 13. WHO should develop a mechanism for rapidly notifying LabNet of important developments such as detection of a new lineage or genotype. These updates could be provided by a List Server or through a “latest news” section in MeaNS or WHO Genotype SharePoint. 

**Action: WHO HQ, Timeline:** First quarter 2011

### 14. Analysis of sequences of the H and P genes of measles in addition to the standard sequencing window in the N can allow finer mapping of chains of transmission. However, this level of analysis will not be necessary in many countries and regions at this time. Some LabNet laboratories will perform the additional sequencing which will form the basis of a LabNet recommendation to describe the situations in which additional sequencing may be necessary. LabNet laboratories are encouraged to share their findings with their colleagues in LabNet and to suggest sample sets that could be used to validate this method. 

**Action:** GSLs, RRLs, WHO LabNet: **Timeline:** Second quarter 2011
15. Quality Assurance
Appropriate positive and negative controls should be run on all RT-PCR assays. Labs should strongly consider using the synthetic positive control RNAs provided by CDC to help identify contamination in PCR assays. Follow up to regional training courses which cover molecular techniques should include testing a blind-coded “practice panel” of RNAs. **Action:** WHO, LabNet. **Timeline:** Ongoing

16. Transport of viruses on filter papers provides an economical mechanism to forward samples to RRLs and GSLs for sequence analysis. LabNet laboratories should strongly consider these methods when sending viral samples. WHO should provide standard protocols. **Action:** WHO, LabNet. **Timeline:** Protocols 4th quarter 2010, ongoing

17. LabNet should develop quality control standards for molecular testing including the use of standard controls, monitoring of assay performance, provision of PT panels for PCR and genotyping as well as methods to evaluate the quality of sequence data. **Action:** WHO, LabNet. **Timeline:** Protocols 1st quarter 2011, ongoing

18. A steering committee should be assembled to review and refines the protocols for accepting and distributing sequence information via MeaNS and the WHO Database. The committee should include specialists from the laboratory as well as epidemiology and bioinformatics. **Action** HPA, HQ. **Timeline:** Committee convened end 2010. Report to the 2011 Global Lab Meeting

19. A systematic review of rash causing diseases found to be non-measles and non-rubella by the LabNet should be undertaken in selected countries and reported to the LabNet. **Action:** HPA, GSLs and Regional Coordinators. **Timeline:** Report Global LabNet meeting 2011