REPORT ON THE 11TH WHO GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK MEETING

David Featherstone
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 2013, the meeting comprised of 11 sessions over three full days, including a breakout of regional specific planning for the remaining 2013 and all of 2014. The main topics to be covered included Global and Regional updates, CRS surveillance, Verification of elimination, Serology and seroprevalence, Molecular methods and EQA, Measles and rubella virus genotyping and New developments.

Meeting objectives
The objectives of the meeting were:
- Review and discuss current status and management of the Global Measles and Rubella Laboratory Network
- To provide participants updates on laboratory issues related to measles and rubella control, including LabNet QA
- To develop strategies to address challenges for MR LabNet

Expected outcome:
- Develop a set of recommendations with implementation timelines to strengthen functionality of WHO Measles-Rubella Laboratory Network

Session 1: Opening
The 11th Global Measles and Rubella LabNet Meeting was opened by Michel Zaffran, coordinator of EPI, who welcomed the meeting participants and provided an insight into the recent re-organization of EPI. A survey of partners and stakeholders was carried out to analyse what WHO HQ EPI’s priorities should be. The results indicated that the role of EPI should include: strengthening of routine immunization; providing more practical support for countries with respect to strategic planning; decision-making; new vaccine support and routine systems; strengthening the Measles and Rubella Initiative and improving data quality to enable accurate and quality data essential to guide the programme. The new EPI structure is now organized in a matrix format with three priority areas:
1. Protect: to strengthen immunization programmes in low performing countries
2. Innovate: to help scale up with innovations
3. Accelerate: to Control, Eliminate and Eradicate Vaccine Preventable Disease with a focus on implementing the new Measles and Rubella Strategic Plan.

As part of the matrix, cross cutting alignments include: to improve quality and reliability of data to drive strategies and to integrate Polio’s activities into EPI, and especially the SAGE recommendation to introduce IPV as part of routine immunization in 120 countries.

Mick Mulders, the new Global LabNet coordinator appointed in late 2012, reviewed the 2012 LabNet meeting recommendations. Most of these were ongoing.

**Session 2**
**Update on the Global Measles and Rubella Programme; Peter Strebel:**

Dr Strebel leads the Global Measles Control Programme at WHO/HQ. He reported that a recent SAGE meeting stated that despite progress, based on current trends and programme performance, 2015 global targets as well as regional elimination targets in EUR, EMR and AFR would not be achieved on time. Coverage has levelled off at 85% and EMR, AFR and SEAR are all below 90%.

A total of 72% of countries introduced routine 2nd measles dose prior to 2012, 7 countries introduced in 2012 and 3 plan to in 2013. Since 2000 there has been a 71% reduction in deaths but it is a challenge to get below this level. Cases are levelling off with 2012 numbers looking lower than 2011 but the numbers are not yet finalized. AFR in 2013 is holding the line.

EMR has seen a resurgence of measles, especially in Pakistan. In EUR, seasonal waves of measles still occur and no major improvement is evident. In 2013 Turkey has experienced a large outbreak. SEAR is looking better and India is introducing a second measles dose of measles vaccine. WPR has seen a reduction in cases but there has been some resurgence in 2013 in China in adults 18-35 years of age, infants and pre-school age groups. It appears that the cases in the routine eligible age groups were missed in the national SIA of 2010. PAHO continues to maintain their elimination status but continues to have importation of cases from other parts of the world.

For rubella: GAVI support for rubella will provide more than $500 million for 2012-2020 and it is projected that more than 1 billion doses of MR vaccine will be given by 2020. This provides an opportunity of being a game changer for measles and rubella control.

There is a need to improve surveillance and reporting of CRS. Countries with the highest burden of CRS are in AFR and SEAR. Globally, only 214 CRS cases were reported in 2011, however it is estimated that 100,000 occur.

To achieve measles elimination there needs to be improved quality of immunisation coverage, uniformly. Routine immunization provides a critical platform for elimination and new tools are required for innovation in programme delivery.

The Laboratory has a key role in providing the specificity of the surveillance programme.
Update on the Global MR LabNet; Mick Mulders:

In 2012, 230,000 suspected measles cases were tested by the LabNet, 119,000 samples were tested for measles IgM with 37,000 found positive. For rubella 103,626 specimens tested and 17,807 found positive. In 2013, until June, 114,448 suspected cases were reported, 37,308 specimens tested for measles IgM of which 12,900 were positive. For rubella, 29,142 were tested for IgM and 3,205 found positive.

The RubeNS database is now up and running with 1000 sequences available, thereby making the WHO database less relevant.

New developments were outlined, including: In-house IgM and IgG QC for general distribution, Molecular EQA, a revision of the accreditation checklist and accreditation procedure is underway, use of seroprevalence for verification of elimination, use of WHO SharePoint for sharing current documents, multiplex serology and point-of-care testing.

Some of the challenges facing the LabNet include: timely investigation and reporting of genotypes for measles and rubella; enhancing surveillance in countries/regions introducing rubella; roll-out of rubella; enhancing laboratory-based surveillance to monitor impact of vaccine introduction; genotyping database, particularly rubella; the need to update rubella nomenclature; data inconsistencies between epi and lab reports; and maintaining expertise.

Plans for 2013 include: roll out of a molecular EQA programme; rubella IgG standardization; seroprevalence guidelines; seroprevalence studies in Myanmar, Nepal, DRC Namibia, and Indonesia. Lab accreditation visits by the global LC are planned for Namibia, Botswana, Ethiopia, China, Australia, Japan and Brazil.

Planned workshops include: rubella for AFR; molecular for SEAR; and Oral fluid for seroprevalence purposes in Bangkok. Regional LabNet meetings will be held in all WHO regions in 2013. The next global meeting will likely be held in September 2014.

Global Update on the Reporting of Laboratory-based surveillance data; Marta Gacic-Dobo:

There are two major sources of measles and rubella data received by WHO HQ. Monthly surveillance data received from the WHO regional offices in an aggregated format, by district and month and annual data through the WHO/UNICEF Joint reporting form from countries through Regional Offices. Regions are using mixed data sets, surveillance and lab, and these are not consistent, with some countries reporting incidences of more than 100%. Of the world’s two largest countries, India is not reporting surveillance data and China is not reporting lab data.

There are considerable limitations to maximizing the analysis of data received from the regions. The recommendation from a meeting in 2000 for regions to send only aggregated data to HQ needs to be reviewed due to the subsequent acceleration in the goals for measles and rubella. The reporting of case based data HQ would allow more comprehensive analysis and timely reporting, globally.

Session 3 Regional Updates, progress and challenges
AFR LabNet Update; Annick Dosseh, Charles Byabamazima:

The challenges in the AF region are: routine immunisation coverage is stagnating in most countries, measles outbreaks are occurring in the face of gaps in immunisation service delivery, shifting epidemiological susceptibility towards older age groups, gaps/delays in resource mobilisation for SIAs and hugely inadequate funding for supporting lab and surveillance. Measles surveillance is often considered as still in “mortality reduction mode”.

Rubella: There is limited data on the epidemiology of rubella in the region, although lab data from testing measles negative cases, it can be seen that rubella is circulating among older children and adults. However, CRS is not well documented and not included in IDSR data collection.

For the Lab coordination: the 2 coordinators for polio, measles and rubella manage 44 labs, and also YF in the at-risk countries. Both staff face a heavy workload.

Labs in the AFR share their lab specific data with AFRO weekly but there are challenges in reporting and completeness. However, timely testing and reporting within 7 days is excellent. Confirmatory testing is well coordinated and performed on 10% of all samples that are sent quarterly to the designated RRLs. Two labs missed achieving the minimum score of the PT but this was possibly due to delays in shipping panels to the labs. One of the labs was not accredited because of failing the PT.

Benin and CIV are still collecting oral fluid samples for surveillance purposes. The main challenges in the region include: delays in reporting, kits expiring at same time in the whole region, limited sequencing capacity available, especially with NICD not performing sequencing since late 2011. UVRI is performing sequencing now, but training has yet to be completed for CIV. Limited funding is available to cover supplies and operations but only 60% of demand will be met for 2013.

AFR, RRL NICD, South Africa Update; Melinda Suchard:

Melinda Suchard is the new head of the Measles and Rubella Lab at NICD and reported that South Africa has stopped testing for rubella in 2013 as they feel they have sufficient data on rubella and introduction of rubella vaccination will not occur in the next few years. However, they are considering looking at antenatal samples to identify susceptibles in the population.

For the regional serological EQA programme NICD performs, Angola is not sending samples. NICD has not received any TSA from AFRO for molecular testing and even though they would like to support this activity, they cannot without financial support from WHO.

South Africa is moving from urine to throat swabs for molecular testing. A national coverage survey is being planned for later this year as the coverage rate of measles vaccine is disputed.

Region of the Americas LabNet Update; Dr Gloria Rey:
All countries in the region had introduced MR vaccine into the routine programme by 2012. In the past year, measles cases were detected due to importation events in many of the AMR countries. Ecuador experienced an almost a year-long outbreak of measles with cases detected for 51 weeks from the start of the outbreak in 2012.

Five surveillance performance indicators are monitored in AMR with just reporting timeliness (within 4 days) related to lab. All indicators exceed the 80% minimum in 2013 and the lab has met their timeliness indicator for the past 6 years.

The MR LabNet now includes 135 labs. This follows the MoH of Equator making a decision to test only in the NL after the outbreak, thereby reducing the subnational labs by four.

Avidity testing is only available in Canada and USA in the Americas. Molecular capacity has increased through training activities with the support of CDC. Twenty labs were trained and some are performing sequencing. Accreditation of 9 labs will occur during 2012-13

EQA programme: The Americas' LabNet uses the Global PT programme and 4/5 of the countries with SNLs have also introduced a SNL EQA programme. A total of 10/21 NLs perform confirmatory testing for both measles and rubella, with most of these labs meeting the minimum 90% accuracy required.

Genotypes found for both measles and rubella show a pattern symbolic of importation. More than 100 viruses were sequenced in each of 2012 and 2013 and all were indicative of importation with the European region being the most common source of likely infection. Four countries reported viruses to MeaNS in 2012.

Rubella
Twelve rubella cases were detected in 2012, 9 of them due to importation and the other 3 of unknown origin, but also likely to have been imported.

There is a need for enhanced testing in the elimination phase to prove whether positive IgM cases are true or false positives. There is a need for avidity testing, IgG and genotyping to confirm whether cases are due to importation or not.

The challenges were reported as being: Maintaining the commitment to obtain adequate specimens for viral detection and genotype identification of each outbreak; Improving the analysis and investigation of special cases, following the PAHO Laboratory Guidelines; Improving the implementation of the new molecular techniques for detection and genotyping of measles and rubella viruses; Facilitating the participation of private laboratories in the National LabNet; Improving the effective communication between epidemiology and lab teams to maintain the quality and integrity of the surveillance system.

It was reported that increased financial support for the Lab Network is highly desirable.

The EMR LabNet Update; Dr Hinda Ahmed:
Reaching the Eastern Mediterranean region’s 2015 elimination target will be a challenge due to the political uncertainty in many of the countries and subsequent security issues. For example, in Syria, MCV1 coverage has declined from 78% to 35% in the past 12 months. Many countries are close to elimination but Syria has more than 30 cases in 2013 and Jordan has detected cases in nationals after 3 years of no cases. There are 300,000 refugees currently in Jordan, however, there are plans to focus on the immunization of these groups.

Afghanistan, Pakistan, Sudan and Somalia currently have large outbreaks Most of cases are aged 1-4 years and are mostly unimmunized. Somalia has experienced large outbreaks but these are not reflected in case-based data reported to EMRO.

Sentinel surveillance occurs in South Sudan and Somalia. Case-based measles surveillance occurs in all other countries in the region.

Measles outbreaks are putting a heavy load on the LabNet and though the recommendation is for labs to test only 5-10 specimens per outbreak the surveillance people want the lab to continue to test so that the momentum of surveillance is not lost.

In 2012, 1544 cases of rubella were lab confirmed in the region with a further 63 epi-linked. The Tunisian outbreak in 2012 contributed most of the cases reported in the region and the age distribution identifies most cases in the 5-14 age group.

Most countries are meeting the adequacy of investigation as part of the surveillance indicators for measles. The majority of countries are reporting results within 4 days except for Pakistan (due to the large numbers being tested) and Djibouti. Djibouti and UAE labs did not report the global PT results but the rest of countries met the minimum score of 90%.

Genotyping: FTA cards have been used for shipping samples to sequencing labs for molecular epidemiological purposes.
Measles genotype B3 has been predominant in the region replacing the D4 that predominated before. Rubella molecular surveillance is still weak.
The measles and rubella focal point for the regional office has not yet been replaced and the lab coordinator has been required to cover Rota and PBM surveillance as well as measles and rubella.
A consultant training workshop is planned to provide experience for consultants to cover the staff shortage in the RO.
The chief challenge in the region is the security issues in many of the countries in the region. However, all member states in the region have committed to measles elimination.

The EUR LabNet Update; Myriam Ben Mamou:
The EUR has an elimination goal of 2015 for measles, rubella and CRS. Only one country is reporting less than 80% routine coverage but many countries are not reporting at all.

Most of the rubella cases in the region were contributed by the outbreak in Poland, with Romania and Ukraine also contributing.

Measles age distribution identified most cases >15 years, with the majority of cases occurring in the unimmunized or with unknown immunization status.

In 2013, more than 2000 measles cases were identified in Turkey.

The performance of EUR LabNet in 2012 identified that 66/67 labs were considered accredited, all labs passed the global PT and 48,000 samples were tested during 2012.

Measles genotyping identified D4 and B3 viruses predominant in 2012 with D8 predominant in 2013.

The challenges in the region were identified as: Genotype information is available for only 35 of 53 countries in the region between 2008 and 2013; Reporting timeliness of genotypes needs to be improved; Timeliness and completeness of reporting data to WHO needs improving; Low workload in Eastern European countries; Too many laboratories in the region to visit regularly; Linkage of virological with epidemiological data needs improvement; Standard protocols for molecular methods are not always used; Ensuring financial sustainability of the LabNet.

**SEAR LabNet Update; Sirima Pattamadilok:**

The South East Asian region has newly agreed on a goal of Measles and Rubella elimination by 2020. The SEAR measles and rubella laboratory network was established in 2003 with eight labs. The region had 23 labs in 2012 and saw an increase to 36 in 2013 with the development of 13 Sub-National Labs in Thailand, which are overseen by Thailand NIH. All labs passed the global PT, however, 2 labs did not report in 2013.

Submission of sequence and genotype data to MeaNS and RubeNS improved in 2011 and 2013, respectively.

A total of 17 labs have been accredited through onsite visits, with 13 meeting all the performance requirements and 6 have pending status.

The challenges in the region include: Development of proposed new labs for India and Indonesia; Regional measles and rubella case-based surveillance will start on 1st January 2014; To establish and maintain the high-quality of laboratory based surveillance systems; To ensure all countries implement and report core indicators for monitoring quality of field and laboratory surveillance; To accumulate genotype baseline data of measles and rubella endemic strains for SEAR.

**The Development of a Measles Sub-National LabNet (SNL) in Thailand; Atchariya Lukebua:**

Thailand has established 13 regional medical science centres (RMSC) covering the whole of country following the regional agreement to achieve measles
elimination by 2020. Staff from each of the 14 labs were trained at the national lab in NIH and tested with unknown samples after returning to their own labs. The SNLs test suspected cases from their respective provinces for measles and rubella by IgM EIA. Throat swabs are shipped to the NIH laboratory for culture and PCR for molecular epidemiology purposes. All labs passed the measles test but 2/13 labs missed the rubella PT test through using suboptimal sample volume. NIH has established a programme for following-up the quality of the SNLs. More than 91% reported within 4 days but a target of 48-hour turnaround has been established.

The Development of the Measles LabNet in India; Dr Sashi Kare:
A total of 10 laboratories constitute the measles laboratory network of India. KIPM, Chennai is the designated reference laboratory for confirmatory testing and training. NIV, Pune is the reference laboratory for sequencing. Seven laboratories in different geographical locations serve as national laboratories and provide serological diagnosis of measles suspected cases identified through outbreak based measles surveillance programme. One laboratory (GMC, Guwahati) is in the introduction phase and is currently testing samples in parallel to another accredited measles lab in India. It is expected that the network will expand to another 3-4 laboratories to support measles surveillance program across the country. A system for specimen shipment to an accredited has been established if a state does not have a lab.

Western Pacific Region LabNet Update; Youngmee Jee:
The WPR has twin goals of measles elimination and Hepatitis B control by 2012, with an additional accelerated rubella control to achieve and maintain control of rubella and prevention of CRS. The target year has yet to be set for the rubella goal.

Achievements towards achieving the measles goal have seen a 93% reduction of cases between 2008-2012 with most cases currently under 1 year of age and not vaccinated. Both MCV1 and Rubella vaccine coverage exceeded 95% in 2012. Routine MCV2 is implemented in all but 2 countries, Lao and PNG. However, there has been a resurgence of cases in China in 2013, with an age shift to very young children and young adults. Nosocomial infection of measles has been reported in both RO Korea and Japan.
The regional measles incidence rate was 5.9/million in 2012, its lowest ever, but 23.5/million in 2013 (to June).

The WPR LabNet is a performing at a high level. Most network labs (48/50) were WHO accredited as of June 2013. Molecular capacity has been strengthened through hands-on training workshops in 2009, 2010, 2012, utilising Hong Kong RRL as a training venue and through annual training in China provided by China CDC, supported by WHO and US CDC.

China has an ambitious plan to expand real-time RT-PCR capacity into the 331 prefectoral labs in 2013. The sharing of genotype and sequencing information with the WHO databases has improved, but timeliness of reporting could still be improved. For quality assurance purposes, confirmatory testing occurs 1-2
times per year depending on the lab's workload. Proficiency tests were successfully completed in 2012, and 51 labs participated in the serology PT and all but 2 gained 100%. For the molecular PT, 41 labs participated (11 national labs + 30 China provincial labs) in 2012.

Some of the challenges for the LabNet in the region include: Low laboratory confirmation rate and weak surveillance, especially in Laos and Viet Nam; In some countries such as Singapore the testing algorithm in the NML does not allow measles IgM testing of rubella IgM negative cases; Private sector commercial labs are involved in testing, especially in Australia, New Zealand and Japan which are not quality checked by the LabNet; Discrepancy of surveillance and laboratory data in Australia, New Zealand and Singapore. The receipt of regular lab-based data from China is a challenge, although the sharing of molecular data with WHO is excellent.

New developments in the region include: The region is moving towards a 4 day turnaround for reporting results of IgM testing; Oral fluid collection to improve molecular surveillance, especially in the Philippines and New Zealand; Incomplete clinical and epidemiological information for result interpretation and further testing requires closer communication with EPI/surveillance group.

Session 4: CRS surveillance

WPRO experience; Youngmee Jee:

Lao PDR and Solomon Islands will introduce routine RCV in 2013. Three countries will conduct MR campaigns in 2013; Cambodia, Vanuatu and Viet Nam and PNG will introduce RCV by 2015. Following the large outbreak of rubella in Viet Nam 2010-11 the country decided to introduce MR vaccine, supported by GAVI. They will establish CRS surveillance in 3 surveillance sites in the country. Following the earlier outbreak, 436 suspected CRS cases were detected, 286 (66%) were lab confirmed and 11 clinically confirmed.

CRS surveillance in Japan; Yoshio Mori:

A rubella outbreak began in Japan in mid-2012 and is currently on-going with more than 10,000 cases in 2013 alone (incidence rate 79/million) with CRS a concern. Most (77%) cases are males but more than 1500 cases have been found in females in the child bearing, 15-40 year age group. CRS confirmation is based on Lab and clinical evidence. All pregnant women in Japan have the availability for a health check-up, including Rubella antibody measurement. However HI and IgM are traditionally used if a woman presents with a rash. Eleven infants with CRS were reported between January 2012 and June 2013 in Japan. CRS surveillance will continue. A report on the outbreak was published in MMWR 62(23), 2013 entitled, National wide Rubella Epidemic – Japan, 2013.

CRS Surveillance in the Americas; Marilda Siqueira:

11th Measles and Rubella LabNet Meeting Report; DRAFT 2, 18 Dec 2013
The Region of the Americas has had a very small number of rubella cases reported since elimination was declared in 2010. In 2012, 12 cases were confirmed in Canada, 7 in the USA, one in Colombia and two in Mexico. Eleven countries in the region are reporting suspected CRS cases to PAHO. In 2009, the last cases of CRS in Brazil were reported and their current surveillance notification rate exceeds 1/100,000 of live births with >3/100,000 achieved over the last 3 years.

**CRS report, USA; Joe Icenogle:**
There is no endemic circulation of Rubella in the USA. The three CRS cases detected in the USA in 2012 were classified as imported as the mothers were reportedly infected in Sudan, Nigeria and Tanzania.

**CRS studies in Romania and Brazil; Joe Icenogle:**
The study was outlined as investigating vascular damage in CRS cases and looking for further markers of CRS in women.

For markers of CRS cases, RT-PCR is very useful, virus can be cultured for 6 months and IgM detected for 12 months. Detecting markers for longer periods would be useful for capturing further cases such as alpha fetoprotein (AFP) as a non-specific marker of foetal damage. Also wild rubella virus persists in endothelial cells.

During the Romania outbreak, six CRS cases were confirmed. These cases will be looked at for virus persistence in endothelial cells.

A Brazil CRS study will also be looking at markers to extend the diagnosis of CRS. C protein levels in 6-12 yr old children are much higher than in mothers and are significant as a diagnostic marker, both in sensitivity and specificity, especially if normalized with normal antibody levels.

**Session 5: Verification of elimination and the role of the LabNet**

**A Global Perspective; Robert Perry:**
Verification of elimination depends on multiple lines of evidence and for many of these lines of evidence, National labs, supported by the LabNet, play a key role. This role includes: Surveillance, through classifying cases and meeting quality indicators; All countries with an elimination goal must have access to an measles-rubella laboratory accredited by LabNet, with on-going quality control; Documenting pre-elimination genotypes and verifying absence of endemic transmission through WHO standardised, molecular surveillance; Providing prove of population immunity through methods such as serosurveys. Some of the needs for the future include: Guidelines for cases with equivocal results and sorting false positives from importations; Timely and complete reporting from country to region, to HQ; Data for calculation of key indicators, including age distribution; Reconciliation between epi and lab databases; Need to link lab and epi in tracing viruses/viral genotypes, including possible wider sequence window to help identify virus transmission pathways.
Regional Perspective of Verification:

The Americas’ Verification process; Marilda Siqueira and Gloria Rey:

The Americas are well advanced in their verification process and the LabNet has a critical role to play. It is essential that all laboratories in the Region be fully accredited by WHO. It is planned to complete a “paper” accreditation of all PAHO laboratories by the end of 2013 using the WHO updated accreditation checklist. It is planned to have 14 site visits of key laboratories by the end of 2014. The Regional Laboratory Coordinator (RLC) will explore use of IHR protocols to facilitate shipment of samples within the Region. All laboratories have been requested to submit sequence data to MeaNS and RubeNS or GenBank in a timely manner. It is required for National laboratories to be responsible for monitoring the quality of all sub-national laboratories in their country and they are strongly encouraged to provide a proficiency testing programme for them.

WPRO’s Verification process; Dr Youngmee Jee:

The Measles verification process in the WPR has been based on the polio certification process. Progress to date includes, the formation of Regional and National commissions, with the National commissions presenting documentation to the Regional Commission. WPRO has carried out two regional meetings, in 2012 and 2013, to guide the process. The verification of measles elimination for different countries will proceed at different timelines. Verification for the Western Pacific Region only becomes possible as a result of a successful verification process being accomplished for all WPR countries and areas.

The Lab performance indicators and targets for verification include: Proportion of measles network laboratories that are WHO-accredited for serological and, if relevant, for virological testing (target: 100% of labs); Proportion of serological results reported by the laboratory within 4 days of receiving the specimen (target: > 80%); Proportion of laboratories (government and private) that conduct measles diagnostic testing that have adequate quality assurance mechanisms in place (target: 100% of laboratories); Proportion of virus detection and genotyping results (where appropriate) that are completed within 2 months of receipt of specimen (target: ≥ 80% of specimens received); Complementary evidence includes: completeness and timeliness of monthly reporting (including zero reporting) to the WHO Regional Office for specimens received for serological and virological testing (target: ≥ 80% of specimens received in the laboratory).

EURO Verification process; Dr Myriam Ben Mamou:

EURO has developed a draft plan and the lab has a critical role to play, including: Documenting the transmission patterns of circulating strains of measles and rubella; Identifying endemic viruses and the potential sources of imported viruses; Ascertaining whether elimination has been achieved by documenting
the interruption of transmission of endemic viruses; To be fully integrated with epidemiological case-based data. Some of the challenges anticipated in the region are: Timeliness of sample collection; Systematic testing of all samples; Genotyping baseline being identified, particularly rubella; Financial sustainability of maintaining the LabNet; Continuing outbreaks; Integrating case-based surveillance (epi/lab).

Session 6a- Serology

An update on a Multiplex serology assay for MR LabNet; Fiona van der Klis and Rob van Binnendijk, RIVM:

The Netherlands has introduced a multitude of vaccines in the National Immunisation Programme and there is a need to monitor immunity and disease burden. RIVM has developed several multiplex assays based on the Luminex platform with up to 18-plex assays being developed so far. Multiplexing greatly reduces technician time and speeds result generation with about 30x gain in efficiency with respect to FTEs. RIVM is currently investigating using Luminex for avidity testing and are planning to find the correlation of IgG antibody with PRNT.

Rob van Binnendijk reported that measles surveillance in the Netherlands uses finger prick blood as well as oral fluid. RIVM uses a Protein Microarray system that can use picolitres of antigen added to glass slides. Currently it has been validated for measles and rubella. Luminex detection of measles IgG antibodies has been found to be more sensitive than EIA and early results show good agreement with PRNT results on the same cases.

Point of Care (POC) assay for measles IgG and IgM; David Brown (Dhan Samuel, David Featherstone)

Public Health England (PHE) has been comparing alternative collection devices to the Oracol oral fluid collection device. They have identified some matrices that have a greater potential than the current Oracol sponge for increased absorption and elution. Devices for collecting and extracting were also investigated and will be assessed in an on-going field study in Uganda as part of a Gates supported project. A rapid POC test is being developed using Lateral flow technology using capture technology for detecting IgG and IgM for measles, initially. Lateral flow device readers have also been investigated for enhancing the sensitivity of the POC devices. Bill and Melinda Gates’ foundation are supporting a project with a number of groups, including PHE, to evaluate these tools as a mechanism to quickly and accurately evaluate population immunity in the field.

Tuesday Day 2

Session 6b- Serology/Performance
A Report on the annual WHO proficiency test panel PT 01202; Jennie Leydon:

Samples in the 2012 global serology proficiency test (PT) panel were retested after 14 days at room temperature and no significant change was detected. A total of 217 labs tested the PT panel for measles IgM and 212 labs for rubella IgM in 2012. Most of the labs are using Siemens kits except for labs in China and Russia that use locally manufactured kits. Also the labs in the European region that uses a number of other commercially available kits. Almost all labs are reporting validation criteria but 46% are not reporting their cut-off values. Seven labs had invalid tests. Overall the labs’ maintained their excellent results from previous years and 98% passed the measles test and 99% the rubella. However, there was a lower percentage of compliance for laboratories meeting the 3 criteria of passing the panel result, providing validation criteria and reporting results within 14 days. It was reported that a number of labs had correct qualitative results but quantitatively showed evidence of lower sensitivity. However, often these issues were not evident until the complete analysis has taken place. It is essential that these labs are followed-up with by the Regional Lab Coordinators when evidence of performance issues is detected.

Session 6c: Serosurveillance/Seroprevalence

Seroprevalence studies- WHO perspective - Developing guidelines; Mick Mulders:

The purposes of using seroprevalence studies are to: Validate vaccine coverage estimates; Generate population susceptibility profiles; Identify at-risk populations and determine population immunity gaps. As there are many challenges to performing seroprevalence studies, it is recommended that WHO develops a set of best-practices guidelines for immunization serosurveillance and encourages a standardised laboratory testing methodology for VPD serosurveillance.

Seroprevalence studies as part of the verification process; David Brown:

The usefulness of seroprevalence studies was outlined and the options for carrying these out, including using convenience samples or cluster population sampling. The advantages and disadvantages of these procedures were described. There has been no extensive evaluation of EIAs since the Ratnam et al paper in 1995. The question was posed as to whether there is a need to re-visit these. Using alternative samples (OF, dried blood spots) for seroprevalence studies can be useful but there is a need for calibration and validation for specific assays.

Seroepidemiology can provide valuable data to guide vaccination programmes as issues about the quality of some countries’ reported coverage data suggests that it will have a role in elimination criteria. However, carrying out
Seroepidemiological studies can result in high costs and may not be cost effective when data is already available through routine surveillance programmes. To ensure if the data from these studies is interpretable, a wide range of issues should be considered: What is the question? What numbers are required to power the study? How will the data generated be used? Seroepidemiology gives information at one time point and may need repeating, especially after an outbreak or a change in immunization strategies.

On-going seroprevalence studies; Jim Goodson & Paul Rota:

GAVI has recently committed funding for introduction of RCV in all GAVI-eligible countries, thus, it is anticipated that ~51 countries will introduce RCV by 2018. The strategy for introduction is to implement a wide-age-range catch-up MR campaign targeting children 9 months-14 years of age. Countries that are considered eligible for RCV introduction include those that have achieved ≥80% measles vaccination coverage either through routine or SIA. To assess rubella immunity, identify immunity gaps, and guide vaccination strategies, seroprevalence surveys may be considered following the initial MR campaign. However, to interpret the rubella testing results from the seroprevalence surveys, it would be very helpful to have a standardized test kit with a cut-off that indicates prevalence of antibodies indicating immunity. Using the MicroImmune assay, then all positives and equivocal results during the surveys could be considered as immune.

US CDC is involved in several seroprevalence studies in Asia and Africa and includes the countries: Angola, Bangladesh, Cambodia, China, DRC, Myanmar, Namibia, Nepal and Uganda. The China study is looking at performance of OF samples. In Nepal, OF and a subset of serum samples are being collected for measles and rubella seroprevalence. In Myanmar, serum and OF samples are being used for polio, measles and rubella, seroprevalence in the 1-15 year age group. In Bangladesh, a Hep B survey of children and mother-child pairs is being carried out with measles also added. In the African region, DRC is using archived sera for measles seroprevalence, in Angola, a DHS survey is being utilised for rubella and in Uganda and Namibia, retrospective archived specimens are being used for polio, measles and rubella.

The seroprevalence studies should be carefully considered and protocol development should use using multidisciplinary study teams, including: epi, lab, and statisticians. The decision on sample choice can be between convenience versus representative and it is important to involve the statisticians to avoid sampling errors. Before the study begins, it is necessary to assess the capacity and quality of potential labs and ensure adequate trained personnel are available for carrying out the testing. There should be consideration for the capacity to scale up and any impact increased volume of testing will have on the lab’s routine testing. In assessing the capacity of the lab the impact of extra work involved in retesting and QA/QC should also be considered.
A timeline for carrying out the seroprevalence studies should be developed, especially with respect to the supply of kits in relation to the collection of samples to ensure kits are used within their shelf life and not wasted. Consideration for gaining ethical approval for these surveys is critical and the time it may take to achieve this is factored in to the overall timeframe. The timeframe for carrying out the serosurvey is important in determining the duration of study and reporting timeliness.

**National measles surveillance programme, China experience; Xu Wenbo:**

China is using measles serosurveillance to: Evaluate the quality of routine immunization and SIAs; Identify specific weak counties and/or susceptible age groups; Identify the reasons for re-emergence of measles in some provinces in 2013.

Measles surveillance has been incorporated into China's national guidelines. Subjects are selected from high-risk areas, mobile populations and those areas with weak immunization and/or high incidence of measles. The China National Measles Surveillance Programme looks at 8 age groups, with approximately 30-50 selected per cohort which is adjusted according to the incidence of measles in each province. Preliminary results are detecting weak counties and low antibody levels in some age groups. For example, Hunan province was found to have a lower immunity in 1-4 and >15 age groups. Results from these surveys are used to generate targeted immunization activities in low performing areas. Also evidence of high performance can be identified, such as in Fujian Province where pre and post SIA survey data showed high seroconversion rates.

**National measles surveillance programme, Japan experience; Yoshio Mori:**

Japan has a national programme for epidemiological surveillance of vaccine-preventable diseases. The programme started in 1962 and is conducted annually. The aim is to understand the immunity to, or the prevalence of, pathogens and to further promote effective management of the immunization programme. The Ministry of Health, Labour and Welfare conduct the surveillance programme in conjunction with Prefectural health authorities and NIID.

A measles serosurvey in 2008 was carried out in 23 prefectures in Japan. The survey used the Particle Agglutination test with a titre of 1:16 considered positive and 1:128 considered protective. It was found that the age group most affected by the 2008 measles outbreak was young adults, due to them only receiving a single dose of MCV, or none, as infants and with little or no exposure to wild type measles subsequently. Japan introduced routine MCV2 following this outbreak and used supplementary immunisation of teenagers for 5 consecutive years based on evidence of the serosurvey. The country is now close to achieving elimination of measles.
In Japan, a large rubella immunity gap was identified in the age group, 20-50 yrs, as determined by serosurveys.

In 2013, a rubella outbreak occurred with males representing more than three times the number of females due to vaccination strategy of immunising school girls only, prior to introduction of routine rubella RCV to both males and females.

**Immunity and protection against measles, mumps and rubella in newcomers to Luxemburg; Judith Hubschen:**

A study was designed to examine the antibody status of 406 refugees and asylum seekers between 13-70 years of age arriving in Luxembourg. Results showed that more than 58% were not protected against at least one of MMR antigens. It was found that it was mostly the youngest that needed vaccination, irrespective of geographical origins.

The correlation of serum and oral fluid results was also investigated. For measles: 93% of results were concordant between serum and OF, with most of the discordants found in the serum equivocal range. For rubella, 95% of results were concordant. For mumps, 78% of results were concordant with the discordant range wider than that for measles and rubella. However, there were a higher number of serum equivocal samples for mumps than measles or rubella. It was concluded that oral fluid is a good alternative to serum and that it is an easy to collect, non-invasive specimen suitable for antibody prevalence studies for measles and rubella.

**Experience with measles avidity testing and its role in the WHO programme; Kevin Brown:**

For serum samples, avidity is useful for confirming primary infection in low incidence settings, for determining false positive IgM, and can distinguish between primary and secondary vaccine failures.

PHE evaluated using OF for avidity and found a good correlation with serum with OF samples showing increasing avidity with time after infection as long as low OD samples were discarded. Oral fluid samples were found to be useful for excluding primary infection and distinguishing between primary and secondary vaccine failure, however, RT-PCR may be of more benefit in identifying reinfection.

Reinfections are more likely to be recognised with capture IgM assays and RT-PCR tests and are observed during intense exposure, such as within a family outbreak. Measles reinfections may be infectious but are considerably less infectious than primary infections, probably due to the large concentrations of antibody neutralizing the virus. The UK has not seen transmission of virus from reinfections outside close family situations.

Paul Rota commented that US CDC has documented a 20 year old individual with 2 doses of MMR, who developed secondary measles, and in his occupation as a theatre ticket collector resulted in 4 secondary cases of measles, of whom all had...
2 doses MMR, who then had contact with >300 other individuals from which no further cases were identified. The original secondary case also was found to have had high avidity IgG. However, it was concluded that it is unlikely that reinfection is major cause of transmission of measles in an outbreak situation.

The role of rubella avidity for the WHO programme; Joe Icenogle:

PAHO is using the criteria of IgM negative and IgG positive for ruling out a case of rubella. However, the proposed laboratory guidelines for measles and rubella case classification state: For IgM positive suspect rubella cases, “if only a single serum specimen is available and it is IgG positive, then test for IgG avidity; the presence of low avidity IgG can be used to confirm a case”.

US CDC uses an in-house avidity assay using DEAE with a 30% cut-off for their rubella avidity test and “high” and “low” differentiations rather than “intermediate”, which some commercial assays use. It is considered important for a lab to determine their own standard avidity curve that will be influenced by the immunity of the population being tested and the type of cases being tested. US CDC does not use Western blot for diagnostics.

Rubella IgG standardization; Liliane Grangeot-Keros:

There is at least a 10x difference in IU/ml identified in the same sample tested with different assays for rubella IgG, with the more sensitive assays giving higher IUs compared to less sensitive. There is a chance that samples may give negative or positive results, depending on these assays too. A multicentre study was carried out where preliminary results of 135 samples, tested with 5 different IgG assays, gave positive results ranging from 4.4% to 52% with the reason being due to differing levels of IgG antibody levels and/or qualitative results.

There are new generation IgG assays available which use recombinant protein, sub-unit virus and different formats to EIA. Depending on the type of assay and type of antigen used, different results and interpretations could be made.

Cut-offs for perceived immunity are considered critical, especially with a higher proportion of the population now gaining immunity from vaccination, and thus subsequently having lower levels of antibody.

In France, UK and Germany there is evidence of an increasing proportion of the population with lower antibody levels, especially for those obtaining rubella vaccination rather than natural infection. In the UK, of 3,500 subjects aged 6-49 years, 5% had less than 4IU/ml and 20% had less than 10 IU/ml. However, there is no evidence of increased susceptibility to rubella for those with less than 10IU/ml. However, based on the above evidence there is a need for IgG standardization and a critical evaluation of current assays.

Rubella IgG standardization. How difficult is the task? Joe Icenogle:
The standardization of ELISA tests for IgG to rubella virus is considered a valuable activity in the light of the above information. In developing a study, its design should recognize the known characteristics of immunity to rubella. The reasons for seroprevalence studies versus good surveillance should be clearly articulated and all issues should be considered.

Various governing/advisory groups have collective opinions on measures of immunity to rubella. For example, the US Advisory Committee on Immunization Practices recommendations conclude that detectible vaccine antibody may decline over time but immunity does not. It should also be considered carefully that some countries have achieved and maintained rubella and CRS elimination with minimal reliance on seroprevalence studies.

In discussion, it was asked that if countries are asking whether they have greater than 80% immunity following rubella vaccination to avoid the paradoxical effect, is there is a need for a serosurvey to reassure the programme that coverage and surveillance results are sufficient?

It was considered that equivocal IgG results should be considered positive and rather than specify quantitative results, qualitative results are sufficient.

Different assays measuring antibodies to different epitopes and it is not surprising that different results arise. For the Siemens measles IgG assay, their reported mIU/ml values do not have a strong correlation with PRNT mIU/ml values but the Siemen’s ODs greater than 0.1 cut/off have a good correlation to PRNT values of >120 mIU/ml.

**Session 7**

**Summary of Practice Panel Results from LabNet Molecular Workshops 2011-2012; Paul Rota:**

The result of a molecular practice panel (MPP) programme run by the US CDC over 2011-12 was summarized. The molecular practice panels were distributed to all participants at WHO intercountry molecular training workshops during 2011 and 2012 in WPR, SEAR, AMR and EMR. The practice panels contain FTA discs loaded with lysates of measles or rubella infected cells. The filters are non-infectious, but RNA remains intact (for at least 1 year at 4°C), and RNA can be extracted by standard procedures. Panels can be shipped at 4°C or room temperature and stored at 4°C but it is important to control humidity by using a desiccant or sealing when completely dry. The FTA disks can be used to test RNA extraction, RT-PCR, sequencing and sequence analysis. It is considered that RT-PCR products should be sequenced by laboratories if they are capable of sequencing or alternatively shipped to the appropriate RRL for sequence analysis if they do not have this capacity. The practice panels can also be used for the analysis of real-time RT-PCR procedures.

The summary outcome of 179 panels analysed from all WHO regions, included: The genotyping RT-PCR assays were performed well and almost all laboratories reported the correct results with minimal cross contamination observed in standard, endpoint RT-PCR assays.
Most laboratories correctly identified the genotypes. Results were reported in a timely manner. Distribution of material at training workshops or via Regional Laboratory Coordinators was efficient and no problems of stability arose during shipping. It was considered that lower concentrations of RNA are stable on FTA cards if kept desiccated.

Some of the challenges identified from the analysis included: Some labs were using BLAST to identify genotypes rather than using the more appropriate phylogenetic analysis; The failure of some labs to include all controls, especially extraction controls; Contamination was detected in some labs performing real-time RT-PCR on the highly concentrated FTA cards; Some problems were encountered with shipping PCR products to RRLs, with leakage and failure to recover DNA from the FTA filters; Some labs reported truncated sequences found; There was an increased workload for RRLs in performing additional sequencing for NLs sending product for sequencing; US CDC reported an increased workload in producing and QCing the panels; US CDC reported difficulty in reading files from platforms other than ABI instruments; Instructions for reporting in an appropriate format needs to be improved.

The next steps identified were: Standardized methods to be developed; A take home, practice panel should be included as follow-up for all intercountry training courses focusing on molecular methods.

The proposed Molecular PT panel will be different to the molecular practice panel and is defined in the next presentation.

**Molecular EQA: Final Protocol and Implementation; Paul Rota:**

A Standard Protocol for a Quality Control Program for Molecular Methods used in the WHO Global Measles and Rubella Laboratory Network has been sent to interested parties for comments.

In summary, the molecular QC programme protocol considers that all RRLs and some NLs performing measles/rubella molecular testing must participate. Selected sub-national laboratories that perform molecular testing for measles and rubella may participate in a national program developed by the NL of that country. The quality assurance programme for molecular testing will consist of: Testing a standard molecular proficiency panel (MPT); Meeting all LabNet accreditation requirements for a laboratory performing molecular testing; Performance in the MPT will be assessed during the accreditation review and success in the MPT will be one of the determinants for defining whether a lab is fully accredited or not.

The MPT will cover the level of testing carried out routinely in the lab. If a lab only completes RT-PCR testing then that is all they should complete for the molecular PT. The MPT will consist of FTA samples supplied as pre-punched disks placed in an Eppendorf tube. MPT Panels are likely to consist of 3-5 samples and distributed to labs one a year with a requirement for results to reported to the US CDC within 2 months of receipt.
Initially, panels will be produced by US CDC and sent to Regional Lab Coordinators for distribution. The production of MPTs for SNLs would need to be carried out in the region and a validated protocol would be required. It is proposed that the first molecular panels, for both measles and rubella, will be distributed by December 2013 to all LabNet labs performing molecular testing. Results will be analysed and discussed at the next LabNet global meeting.

**Session 8: Regional Breakout session**

The objective of this session was for the regional representatives to form small groups and discuss Regional achievements and challenges and develop an operational plan for 2013 and 2014. Each of the Regional groups reported back the following day and their conclusions are summarized briefly below:

**AFRO, Plans for 2013-14:**

The challenges identified for the African region include: The lack of adequate funding has affected implementing a comprehensive sequencing programme in the region; Having sufficient human resources to facilitate the procurement of timely supplies for all labs in the region; The need to develop SOPs, and follow-up on completeness of reporting and ensuring that labs allow for inherent delays in the distribution of kits and other supplies provided by WHO; The AFRO “system” for procurement and operating needs to be considered; The management of kit use in the LabNet in relation to workload, especially related to outbreaks and the need to encourage countries to use the recommendation for epi-linking of cases during large outbreaks thereby reducing workload and kit consumption; The need for molecular training of CIV staff at US CDC to build sequencing capacity in the Western and Central bloc; The need for field training and the sensitization of field staff for collecting samples for molecular surveillance purposes.

The future plans for the African region include: Advocating for more funds to support the regional LabNet; Sequencing training for CIV RRL staff at US CDC; Streamlining the funding and administration procedures for supporting NICD in their sequencing of Eastern and Southern countries in AFR; Instigating field training for field staff to appropriately collect samples especially for molecular surveillance.

**EMRO, Plans for 2013-14:**

The challenges identified for the Eastern Mediterranean region include: Reaching the 2015 measles elimination target following the major measles outbreaks in AFG, PAK, SUD and YEM; Many Member States are remaining in disease control mode and not moving to the elimination mode; Completeness and timeliness of national data for monitoring progress to achieving elimination; The need for case-based surveillance for all countries; The introduction of rubella vaccine and the improvement of surveillance to monitor progress;
Establishing CRS surveillance in the region; Assessing population immunity through serosurveys; Laboratory confirmation of suspect cases; Adequate sample collection and transportation; Budget constraints for molecular reagents and building lab capacity; The increasing workload of the laboratory network; Achieving the minimum 2/100,000 investigation rate; Sharing molecular epi information with the region and globally; Intensifying the collection of samples for virus identification; The detection and differentiation of imported cases vs. endemic virus circulation in a timely manner; Countries with large outbreaks continue to test all samples from suspected cases; the need to strengthen the integration of laboratory and epidemiology surveillance; Developing a laboratory testing algorithm for the measles and rubella elimination phase; Four countries are sending data weekly but the RO cannot cope with the data and still report monthly; Considering whether to use avidity testing for confirming sporadic cases from elimination countries; The PT panel is not being tested by all labs and there is a need for the RLC to follow up on any issues detected ASAP.

The future plans for the Eastern Mediterranean region include: An Inter-country meeting, 18-20 November 2013 to be held in Tunis; A Laboratory training workshop to be held in April 2014; Accreditation visits are planned for Djibouti, Pakistan, Sudan, Yemen, Jordan and Bahrain.

**EURO, Plans for 2013-14:**

The challenges identified for the European region include: Meeting the 2015 elimination goal in the face of continual large outbreaks; Issues with multiple reporting systems in place with plans to scale up MR lab LDMS will be an additional burden; Communication, information and operational guidance; Relevance of data requested by WHO and is it really needed by WHO; Delay in the information about outbreaks in member states, especially as reported to the RRL responsible for the labs in these countries; Capacity building needs in some NLs to meet accreditation requirements; Quality of the molecular testing in some countries; The testing and reporting of measles and rubella from Private labs in the region;

The future plans for the European region include: The need for realistic communication about 2015 goals and the clarification of elimination and countries’ accountability for reaching the goal; The contribution of the LabNet in the update of reporting systems; Regular communication and feedback with WHO, RRLs, GSL and NLs; WHO official guidance on genotyping; Accreditation visits and trainings in selected countries, with joint missions of WHO and RRL staff and Regional LC and HQ LC in RRLs and GSL visits; Quality control of molecular testing to be established; RLC to visit RRLs for discussions and to provide support.

**The Region of the Americas, Plans for 2013-14**

The plans identified for the Region of the Americas include: The focus of the region is on the verification of elimination of measles and rubella; Plan for
accreditation of 14 NLs, 6 onsite visits in 2013, 8 in 2014; Development of a team of senior scientists in the region to serve on review teams; Lab reviews to focus on priority countries in the region for the IEC Peru, Argentina, Brazil, Ecuador, Guatemala, Venezuela, Paraguay and Uruguay, these will be in addition to the accreditation visits; Focus on reviewing the negative IgM samples to ensure these are true negatives; Regional meeting for all NLs planned for 22-24 Oct 2013, location still to be decided (Panama, Atlanta); IgM in-house control protocol to be developed based on the proposed standardized IHC; Finalise lab guidelines describing regional testing strategies for additional lab testing for sporadic IgM positive cases and share with the WHO LabNet; Develop a plan to implement avidity testing by the RRLs and develop a referral plan; Consider producing a regional serum panel to allow RRLs to calibrate avidity assays; A molecular methods training workshop to be held in 3rd quarter 2014 (Canada or Mexico); A recommendation for all labs to perform IgG testing, as required; Selected labs to participate in WHO Molecular PT programme; Improving reporting of sequencing data to MeaNS and RubeNS.

SEARO, Plans for 2013-14:

The Polio endgame will start in January 2014 and the focus will then be turned onto measles elimination by 2020 with case-based surveillance starting in Jan 2014. However, countries have different infrastructure and different capacities.

The challenges identified for the South East Asian region include: Ensuring all countries implement and report core indicators for monitoring quality of field and laboratory surveillance; Accumulating measles and rubella genotype baseline data of endemic strains for all countries; 36 labs are in the LabNet but only 20 use the WHO PT programme

The plans identified for the South East Asian region include: A regional workshop on surveillance standards for EPI diseases, 23-27 September 2013; Developing a case-based surveillance system for all countries; Update the reporting system with online submission and feedback; Regional consultation of virologists from the measles and rubella laboratory network, 21-22 November 2013, Bangkok, Thailand; Identifying priorities and developing country and lab network plans; Developing a standardized protocol to establish baseline data for molecular monitoring; Complete accreditation reviews of laboratory network; Support expansion of lab network in the region wherever required; Integrating the polio focus of the Indian LabNet with measles surveillance.

WPRO, Plans for 2013-14:

The challenges identified for the Western Pacific region include: Capturing the whole of the national data related to measles and rubella, especially with the large proportion of testing occurring in private/commercial labs in some countries. However this will involve MoHs endorsement; The role of molecular testing compared with serology as primary diagnosis in some
countries; Trying to get a second sample if an early first sample is collected; Working under high workload circumstances for virus isolation (China); Implementing QA/QC of sub-national labs in China and Japan; Training needs for lower level labs and the role of CCDC and or WHO in supporting this. Some of the challenging non-Lab issues include: Disparity of reported coverage versus real coverage; Surveillance for measles and rubella and how to integrate rubella surveillance; Determining onset of rash and whether this was indigenously acquired or imported from another country.

The plans identified for the Western Pacific region include: Accreditation of laboratories with pending status in 2013; Further strengthening of strain surveillance for measles from all chains of transmission, both sporadic and outbreaks (and rubella) and provide WPRO MR bulletin with monthly updates; Follow-up of 4 day timeliness for testing and reporting of IgM results; Use of Oral fluid collection to strengthen molecular detection in Philippines and New Zealand; Real-time RT-PCR training for prefecture labs in China; Close follow-up of China sub-national labs for QA of molecular detection, including prefectural/provincial labs; Follow-up hands-on lab training in 3rd or 4th quarter 2014.

Section 9: Summary of the Measles Genotypes Detected in the Americas: 2012-13

The Region of the Americas: Alberto Severini, Paul Rota, and Marilda Siqueira:

Seven countries reported measles sequences in 2012 and three in 2013 with all reporting to MeaNS except for Venezuela and Argentina which have yet to report the D4s they found in 2012, and Brazil the D8 detected in 2013. There has been no H1 detected from China for 3-4 years.

In 2013, USA reported 107 measles cases with 100% identified as imported or import related, with 27 separate importations detected. Genotypes B3, D4, D8, and D9 genotypes were detected. Two outbreaks made up 74% of cases; 56 cases of D8 were identified in a vaccine objectors community in New York and 23 cases of D8 were identified in Orange County, also amongst a vaccine objectors community.

The goal of collecting samples for genotyping from 80% of chains of transmission has been analysed. USA could determine genotypes from 96% of outbreaks larger than 5 but 37% from chains of infection of 3 or fewer cases.

Canada reported 23 measles cases found in 2012-13 and genotyped 21 of these which included the genotypes B3, D4 and D8.

Brazil reported 2 cases found in 2012, identified as D4 and D8 and 35 cases found in 2013, which were all genotype D8.
Identifying the source of virus without epidemiological information is becoming more complex with multiple lineages of B3, D4 and D8 being found in many countries in the world.

The Ecuador outbreak has been all B3 genotype and the sequence almost identical over the 51 weeks from first to last case. The sequence was identical over the 450nt of N gene to the 2009 Zimbabwe virus, MVj/Harare.ZWE/38.09. Some minor variants of this strain were also found. A B3 sequence identical to the ZWE virus was also found in Canada and USA, some of which were linked to an importation from Pakistan.

It may be difficult to identify the source of the virus from just the sequence data but sequencing can help in identifying separate sources of infection and point to possible countries or regions of source.

**The African Region; Annick Dosseh:**
Prior to 2011 only NICD in South Africa performed sequencing for the region and they mostly used serum samples from the confirmatory testing programme. Following the interruption of testing by NICD in 2011, specimens from CIV were sent to CDC. UVRI, Uganda was trained in sequencing by US CDC and began testing in 2012. In 2012, B3 viruses were identified in BEN, CIV, Congo, Nigeria and Togo, and a single D4 virus was found in Uganda with an epi-link to the UK. In 2013, only one virus was reported, a B3 from Uganda. The number of samples collected from outbreaks for molecular testing is low and some countries with outbreaks have not collected any samples. The gap left by NICD not sequencing is huge as they were able to sequence serum from those countries where no molecular sampling was occurring. AFRO’s surveillance guidelines require the collection of samples for molecular studies from each outbreak (>5 cases) although this does not happen in many countries. The challenge of a limited sequencing capacity in the region and the cost of molecular reagents has contributed to the paucity of molecular data.

**Uganda; Barnabas Bakamutumaho:**
Sequencing capacity was established at UVRI with support from US CDC. Predominantly B3 virus was found in 2012-13 in Uganda but a single D4 in Kampala was identified in 2012. Samples collected from field staff resulted in a low positive rate but when lab staff collected samples the positivity rate increased. This was accounted to field staff’s poor collection techniques and by them not utilizing the cold chain when shipping back to the lab. UVRI has detected rubella 1E from Uganda for first time (2012), but most cases are 1G. Other countries served by the RRL are not sending samples for molecular detection. UVRI has identified the need for a follow-up visit by the molecular team from US CDC.

**The Western Pacific Region; Youngmee Jee:**
Baseline genotype data has been identified for all countries in the region except for Guam. Regional updates on genotype data are published in the quarterly WPR Measles Bulletin. Outbreaks in the region were identified as H1 in Lao over
2012-13, with all viruses having 100% homology. The New Zealand D8 outbreak started from cases being infected in an international flight to the country and lasted for less than 12 months. Australia has a pattern typical of a country in the elimination phase, with multiple genotypes detected, epi-links to importation and evidence of limited spread. In total, WPR detected 119 imported cases in 2012-13 (June).

Three rubella genotypes were detected in the region. From the 27 viruses genotyped in 2013, 1E, 1j and 2B were confirmed.

Molecular surveillance in Japan; Katsuhiro Komase:
The number of measles cases detected in Japan amounted to >11,000 in 2008, 293 in 2012, 138 in 2013 (week 21). A 97% reduction in 2012 compared with 2008 when MCV2 was introduced with a catch-up. The endemic D5 genotype, which was found in 2006-2009, has not been detected since May 2010. In 2012, D4, D8, D9 and H1 strains were detected and in 2013, D8, D9, H1 and B3 were detected. B3 was found for the first time and the case had a travel link to Thailand. An Australian B3 also had an epi-link to Thailand. Two H1 strains were also identified with epi-links to China. The 2012 D8 outbreak had identical sequences to a case with an epi-link to Thailand. Another D8 case was found with an identical sequence with a Singapore virus.

Most measles cases now found in Japan are considered to be imported or import related, based on epidemiological investigation and molecular analysis.

Molecular surveillance in China; Yan Zhang:
There was a resurgence of measles in 2013 after the lowest level of measles cases detected in China ever in 2012. More than 16,000 cases were detected in 2013, 6.6x more than 2012. The D11 viruses identified from Yunnan province mainly, was found in 2011, but not in 2012 or 2013. In 2012, a D9 was found in Yunnan with epidemiological evidence of importation from Myanmar. Multiple genotypes of measles have been found since 2009 providing evidence of good viral surveillance, but H1 still predominates. In 2012, most transmission chains were interrupted after the SIAs and the circulation of a smaller number of lineages is now apparent. In 2013, 1300 viruses were isolated, most of which were H1 with 28 D8s and 7 D9s also detected. In 2013, seven provinces reported more than 100 measles virus isolates.

D8 was identified for the first time in 2012. One had a North Korean linkage, but others in Beijing had no known links to importation. An outbreak of 6 cases in Shandong had D9 virus confirmed, with epi-links to Myanmar.

The reduction in diversity of H1 is still be quantified following the SIA in 2011 as a measure of success of the SIA. PHE can assist CCDC with the analysis.

Molecular surveillance in the Eastern Mediterranean Region; Hinda Triki, Suleiman Al Busaidy, Talat Mokhtari and Hinda Ahmed:
The improvement in molecular surveillance in the region since 2007 has continued. Lebanon and the United Arab Emirates have reported genotype information in 2013 for the first time. It is now only Palestine in the region that has no genotype information, however, the area has very high coverage and no measles cases are reported.

Prior to 2011, D4 was the predominant genotype of the region and B3 in a small number of countries mostly situated in the African continent. However, since 2011, B3 has been the predominant genotype, identified in 14 of 22 countries, and D4 has diminished, being identified in only 3 of 22 countries.

The B3s identified since 2011 are in a lineage that is different to the previous B3 lineages. Early viruses of the new cluster were first found in Saudi Arabia but these were likely imported although no epi-links were identified. Pakistan is likely to have a Measles SIA approved for later in 2013 following the big measles outbreak in 2012-13. The Syrian measles outbreak is a D8 and there are concerns of spread to other countries in the region with the current refugee movement. The current outbreak in Lebanon has had 3 genotypes identified, H1, B3 and D8.

Iran has high reported coverage and good measles control, however, most of the measles cases found in the country adjoin Pakistan's Baluchistan region that has a very porous border with Iran. D4 was predominant prior to their SIA and subsequently multiple genotypes were found. H1 was first found in 2009, with epi-links to China, and subsequently in 2012, related to the 2009 strain. However, the 2012 H1 belonged to a slightly different lineage. D4, H1, D8 and B3 genotypes were found in 2012 and B3 and D4 were identified in 2013 with B3 predominant in both years.

In 2012, 8 countries in EMR reported measles confirmed cases by IgM but no genotype information reported. Of the highest incidence rate countries, Sudan, South Sudan, Afghanistan, Yemen, Qatar, Libya and Pakistan (incidence of >40/million), all reported B3 virus except for Afghanistan and South Sudan that have no genotype data reported.

There is a need to improve molecular surveillance in the region, especially in relation to specimen collection for virus detection. For example, Pakistan has found B3 but have not sent virus details to the WHO sequence databases. The Pakistan lab's accreditation is pending due to them not meeting this criterion.

**Molecular surveillance in the South East Asian Region; Patcha Incomserb:**

Data was reported from the RRL sequencing lab in NIH, Bangkok where only Bangladesh, Nepal, Myanmar and Thailand have provided specimens for molecular testing since 2011. Genotypes D8 and D9 were predominant over 2012-13 from these countries. India reports their molecular data separately to WHO through NIV, Pune and D8 viruses from India in 2013 were reported through MeaNS.
In 2014, SEARO will introduce cased-based surveillance in the region, including Myanmar, which applied to GAVI for an MR campaign in 2014. Rubella genotype 2B was reported from Sri Lanka, Nepal and Thailand in 2012. No other rubella sequencing information was available.

**Molecular surveillance in the European Region, PHE UK; Kevin Brown:**

PHE has changed their strategy for sequencing in early 2013 to sequencing only new lines of transmission rather than multiple viruses from the same outbreak. In 2012, D8 genotype was predominant (Taunton strain) with some D4 and B3 detected. In Wales, D8 (Swansea) was found but this was a slightly different strain to the Taunton lineage A D8 identified in Shrewsbury was also a different strain to Taunton and Shrewsbury.

**Molecular surveillance in the European Region, RKI Germany; Sabine Santibanez:**

Of the countries served by the RKI over the past 12 months, B3 and H1 genotypes were found in Sweden, D4 was found in Austria, Hungary, Germany, Norway, Romania and Poland. D8 was confirmed in Austria, Bulgaria, Germany, Norway, Romania and Sweden. D8 (Frankfurt Main strain) was widespread in Europe and D8 (Villupuram) was found repeatedly in Scandinavia. The D4 strains, which were predominant before 2013, appears to be being displaced by D8 strains.

**Molecular surveillance in the European Region, Luxemburg; Judith Hubschen:**

Of the countries served by Luxemburg in the European region over the past 12 months, 34 distinct variants of D4 were found, although most were D4-Manchester like. Twenty-five D4 variants were found in Spain, 9 variants in France and 3 variants in Belgium. Seven distinct variants of D8 were found in France, Netherlands, Turkey, Serbia, Georgia, Portugal, Syria (via MSF), Spain, Belgium and Macedonia, and 3 of them are currently very widespread in the EUR and beyond. B3 and H1 were also detected with B3 found in France and the Netherlands, and was the cause of the Turkey and Israel outbreaks. H1 was found in Portugal with epi-links to China.

**Molecular surveillance in the European Region, Russian Federation; Sergey Shulga:**

In 2012, the Russian Federation confirmed 2130 cases of measles, an incidence rate of 15/million. Resurgence of endemic measles in the region in 2010 – 2013 was due to prolonged local transmission of imported viral lineages which included: D4 (MVj/Bandarabas.IRN/05.10/2) in Uzbekistan, Kazakhstan, Kyrgyzstan and Russia; D4 (MVs/Manchester.GBR/10.09) was detected in Ukraine. Multiple importations of different genotypes and lineages in Russia were detected in 2013. Genotypes D4, D8 and B3 were identified with local spread.
evident. The previously predominant genotype of D4 was replaced by D8, as similar with other European and Eastern Mediterranean countries. Tracking the virus transmission pathways for the genotypes D8, D4 and B3 has proven to be challenging.

**MeaNS Update; Richard Myers, Kevin Brown:**

The MeaNS sequence database currently consists of 12,177 N450 sequences and 606 H sequences. EUR countries are the largest contributors by number of sequences submitted and the number of unique users has been increasing by year. Genotypes D4, B3, D8 and H1 are the most commonly submitted sequences overall with D8 the most common. Currently, D8 is represented by 4 major clusters, Taunton, Villupuram, Frankfurt Main and Swansea, however, multiple lineages of D8 exist in Europe probably as a result of multiple introductions. The MeaNS database is working reasonably well and an update of WHO name checking is on-going. The summary data reported to regions has found to be useful at the regional level. Contributors requested if they could download summary data (non-sequence information) from MeaNS and this would be looked at for making it easier. Mapping and geographic details will be discussed at the MeaNS/RubeNS steering committee meeting on 27 June 2013. There is a future need for adding better resolution, including a larger sequencing window and a more defined geographic location to try and determine the significance of variants.

**Extending the Measles Genotyping Window; Alberto Severini (with contributions from Kevin Brown, Richard Myers, Judith Hübschen, Sabine Santibanez, Paul Rota and Marilda Siqueira):**

The 450nt N gene target is practical and effective for distinguishing MeV genotypes, but it can remain stable for years within each outbreak genotype as for example the genotypes D4, D8 and B3. Definitive tracking of MeV strain spread and importations is not always possible on the basis of the N450 sequence alone. In elimination countries, documentation of the interruption of endemic transmission may become challenging and developing alternative genotyping targets could improve genotyping resolution. The sequencing of H and P genes has been shown to provide better resolution For example; almost all the B3 strains from Ecuador have the same N450 sequence. Also the N, P and H sequences individually don’t show much difference but the combination of these show lineages of which most have epidemiological linkages. For the D4 Brazil strains, H sequences provide better resolution than N alone and indicate multiple importations. The P and H gene sequencing of D4 (Manchester) in Europe showed epidemiological clusters and most of the geographical clusters had a temporal relationship, and sometimes showed transnational circulation. P and H genes appeared to have approximately similar contributions to overall variations and some evolution was evident during circulation, as the strains from late collection time points appeared more distinct.
The D4 (Manchester strain) outbreak in Canada had a total of 776 cases reported from Quebec in 2011, of which 118 isolates were available for genotyping, all of which were identical for the N450. The H gene of these mostly showed some clustering also. The whole genome sequence of 86 cultured viruses showed clades comprised of closely related sequences clustered over the same time period. In the mapping of mutations, a greater variation was found in the M/F coding region. However, it is technically challenging to sequence this region due to its high GC content. In conclusion, the current N450 target is insufficient for differentiating closely related outbreaks. Sequencing of the H and P genes improves the resolution of outbreak phylogenies, but only to some extent. However, the longer the sequence, the better the resolution. There were discussions on whether a new genotyping target would provide more useful molecular epidemiological information, with the caveat that this would involve some extra costs and time and the LabNet should consider the circumstances that warrant the extra resolution an extended genotyping window provides. It was considered that ultimately the whole genome sequencing might be required to document interruption of endemic transmission.

**Session 10: Genotyping Rubella and RubeNS**

**Global update on rubella genotype distribution and nomenclature; Joe Icenogle:**
A summary of the key issues in the pending WER Rubella Update was described. In summary, provisional genotypes 1h, 1i and 1j are upgraded to accepted genotypes 1H, 1I, and 1J. Genotype 1a is the sole remaining provisional genotype. Viruses of genotypes 1G and 2B show significant genetic diversity (2.6% and 2.4% respectively) however, more precise subdivision of these genotypes would improve the ability to track these viruses. Modifications to the virus naming conventions will be described and the RubeNS database will be described. Epidemiological investigation and molecular evidence are both important to identify the source of virus and for the detection of importation. Globally, over the period 2011-12, the 4 most common genotypes reported were 1E, 1G, 1J and 2B, as expected. Only 3 sequences have been reported to the WHO database in 2013.

**Rubella in the European Region, UK, 2012-13; Kevin Brown:**
More than 1500 samples were tested and only 5 rubella cases were confirmed and all were non-UK born. None was sequenced over the 739nt, however, two 2B genotypes were identified from the small fragment sequencing and were from Poland-linked cases.

**Rubella in the European Region, Russia; Sergey Shulga:**
The Russian Federation introduced case-based surveillance for rubella in 2011 after rubella incidence reached below 1/100,000. In 2004-2010, 1H genotypes predominated and this genotype was considered endemic in the country. Since 2010, no 1H has been detected, however, 2B genotype has now become predominant. Two clusters of 2B viruses were identified, one linked to an importation from Viet Nam and the other from India or Romania. Two clusters
of 1E genotype were found, one sequence found in 2012 was related to viruses previously found in the Russian Federation in 2008 and one cluster from 2011, which was imported from China. The Russian RRL reported identifying 739nt genotypes from about 1% of seropositive cases.

**Rubella in the Western Pacific Region, China; Xu Wenbo:**
In China, the highest incidence of rubella occurs over the Spring months, March to June. The highest incidence of rubella occurred in 2008 with more than >120,000 cases detected, however, numbers have declined since. In 2012, the lowest number of cases were detected, 40,362, with 6/31 provinces reporting incidence of <1/100,000. The age group distribution of rubella cases shows that the proportion of the 15 to 39 year age group infected has increased since 2004 when they made up approximately 20% of all cases and remained at about 40% from 2007 to 2013 (May). Three CRS cases have been lab confirmed, 2 in 2011 and 1 in 2012. Two genotypes, 1E and 2B have predominated in China for the past decade. In 2012, 1E was found in 18 provinces and 2B in 10 provinces. These viruses have not yet been compared with the viruses found in the Russian Federation which were considered China imports. Two lineages of 1E and 3 lineages of 2B genotypes have been found. Lineage 1 of the 1E has not been identified since 2008. The China LabNet is successfully using FTA cards to transport virus from the Provincial Labs to the National Lab.

**Rubella in the Western Pacific Region, China Hong Kong; Janice Lo:**
Most rubella infections in Hong Kong occur in adult males as a result of the country focusing on the immunization of women of child bearing age (WCBA). The country uses serum and throat swab samples for virus detection. Genotypes 1E and 2B predominate in Hong Kong, with 1J viruses detected in a few cases. The number of rubella cases by year, were; 2011 (84), 2012 (44) and 2013 (13), with 3 CRS cases confirmed in 2012. The Hong Kong lab uses a nested RT-PCR and use overlapping fragments for the 739 nt fragment.

**Rubella in the South East Asian Region; Patcha Incomserb:**
Only 5/11 countries in SEAR are sending samples for rubella virus detection, with Nepal sending almost 50%. Genotypes 1E and 2B were found to be circulating in these countries, with evidence of 2B in Nepal, Sri Lanka and Thailand, and 1E in Sri Lanka and Thailand. However, the success rate for rubella virus detection is low from these countries. The RRL is not using FTA cards for shipping samples or PCR products.

**Introducing RubeNS; Richard Myers:**
Development of the Rubella nucleotide database (RubeNS) has progressed since the last meeting and a live site is up and running but is still undergoing further development. RubeNS currently contains around 940 samples of 739 bp sequences. A live demonstration of RubeNS capabilities was given, with the procedures for registration, data entry, tools for exact matching, BLAST and genotyping described.
It is considered that 1a genotype has multitude of lineages that cannot be considered a logical phylogenetic cluster. The viruses currently in this cluster need further work to be accurately classified. It is considered that some outlying sequences in genotypes, other than 1a, where the tool cannot reliably identify the correct genotype.

Most of the sequences identified recently, predominantly, belong to genotypes 2B, 1G and 1E.

Although the website is up and running there are still areas under development. Stability, interaction with GenBank and its functionality are still not optimal. All participants were encouraged to use RubeNS and provide feedback to PHE for fine-tuning its performance.

**Session 11: R&D**

**Introduction of New or Improved Technologies for LabNet**

**New and Improved technologies; Paul Rota:**
**Microneedles for vaccine administration:**
Cotton rats have good immune response to measles vaccine virus on microneedles. Dissolving needles were trialled in rhesus monkeys and all had detectable IgM antibodies to measles at day 14 post-vaccination, and neutralizing antibody was found at 28 and 35 days post-vaccination.

**Freeze dried FBS:**
US CDC has evaluated the new commercially available freeze-dried Foetal Bovine Serum (FBS). This product was reconstituted in distilled water and appeared to have the same qualities for supporting cell and virus growth as liquid FBS. SLAM expression in VeroSLAM cells grown in freeze-dried FBS was the same as for liquid FBS. The advantages for shipping without dry ice and the convenience of not having to store at -20C is considerable and likely to save the LabNet valuable resources, however, the cost/benefit data is not available.

**Improvement to Real time RT-PCR:**
The purpose of the proposed study is to compare the sensitivity of RT-qPCR assays used for detection of measles and rubella in LabNet laboratories. Several, different RT-qPCR assays for measles and rubella are used in the LabNet, however, because the methods for establishing the lower limits of detection vary between assays, it is difficult to compare the assays based on data generated during routine surveillance. Knowledge of the lower limit of detection of the frequently used assays could help improve capacity and direct expansion of molecular techniques in LabNet.

FTA cards stabilize RNA and inactivate measles and rubella viruses. RNA dried onto FTA cards was found to be stable at room temperature and could be extracted from the cards and used in RT-qPCR assays. It was considered that FTA cards could be an inexpensive and safe way to transport RNA samples.

**Improving the survival of VeroSLAM cells in the deep freeze; Yoshio Mori:**
A proposal to remedy the problems of low recovery rates from Liquid nitrogen of VeroSLAM cells and also address the problems with CITES restrictions for the distribution of African Green Monkey cells was discussed. NIID is planning to establish a novel cell line that is sensitive to the culture of measles and rubella virus and can still address these issues.

New generation sequencing; Ion Torrent; Claude Muller:

Luxemburg analysed H and P genes from original swabs of 3 patients with >150,000 individual viral genomes for each patient, with a coverage of 0-150,000 for the different nt positions using 4 amplicons in Ion Torrent sequencing. Amplicon sequencing yielded very high coverage and quality reads of >150,000. It was considered that data analysis required stringent QC and did not easily lend itself to be automatized. Experiments and analysis can be done in-house: €480/patient using a 318 chip, 400bp kit. Cost reduction is possible by multiplexing, although the maximum number of samples needs to be determined by required sequencing depth. A comparison of Sanger versus Ion Torrent sequencing by referenced-based mapping revealed no difference within the sequences, and no sequence variability was found in the H and P gene within one patient’s sample. Errors of Ion Torrent are; typically insertions/deletions, however these are rare in MeV and rarely, substitutions, which are frequent in MeV. It was concluded that Ion Torrent is a good choice for deep-sequencing of MeV

Structural model for epitopes on MeV H, Functional Constraints on the Measles H Protein Prevent Escape from Neutralization; Katsuhiro Komase:

Eight distinct recombinant MeVs (rMeVs) encoding a luciferase reporter gene and an H gene derived from different MV genotypes were used as neutralization targets. While B5, E81, E103, and 2F4 neutralized all of the MeV genotypes, E128(II) was only effective against genotypes A, B3, D8, and H1. The neutralizing titres of E185 and E39 were significantly lower than those of other mAbs. These data suggest that antigenic sites I, II, vi, and vii are effective neutralizing epitopes, and that, with the exception of antigenic site II, all epitopes are conserved among the different MeV genotypes.

Neutralization by sera from measles vaccinees and measles patients against rMeVs harbouring escape mutations was analysed. Three additional rMeVs (genotype D3) featuring the escape mutations Q311R and Q391R (Q311R/Q391R), Q311R, Q391R, and R533A (Q311R/Q391R/R533A), and Q311R, Q391R, and D505S (Q311R/Q391R/D505S) were generated. These viruses replicated less efficiently than the parental virus in cell cultures, and both triple-mutant rMeVs completely lost SLAM-binding activity. Only 2F4 mAb neutralized the Q311R/Q391R virus, and none of the tested seven mAbs neutralized the triple-mutant rMeVs. Nevertheless, sera from vaccinees or measles patients neutralized these rMeVs as efficiently as parental MeV.

Revision of WHO Accreditation Checklist; David Featherstone:
The LabNet Accreditation checklist was reviewed as part of the regular periodic review process and to pre-empt the requirements of the Verification committees monitoring progress towards and achievement of elimination. It also includes the introduction of new molecular PT programme and strengthens other QA/QC activities as well as clarifying some ambiguous language.

The details of the new draft checklist was described and included: Recording of BOTH 4 and 7 days reporting timeliness for IgM testing for M&R; Results and timeliness of reporting new molecular PT; Reporting timeliness of sequence data to the MeaNS and RubeNS databases; Country specific surveillance summary data (new Part IV); Lab test summary results; (Pos, equiv, Neg); Separate checklist for National labs that support sub-national labs.

The new RRL checklist will include: A table summarising National Labs validation samples; The onsite review component will now be Part V; An expanded lab management section will be included in the Onsite review part.

New reporting timelines are proposed for the accreditation assessment. Annually: All Laboratories will be required to provide complete data for Parts II to IV of the new checklist. By the end of January the following year: Regional Coordinators will provide a summary of the performance indicators to the Global Lab Coordinator for all labs by the end of February of that year. The Global Lab Coordinator will provide a summary of the global lab accreditation results by the end of March for the WHO annual report and to be published on the LabNet SharePoint. Onsite reviews of labs will follow the same pattern as before, every 1-4 years, depending on the performance of the labs, with the high performing labs being visited less frequently.
Meeting Feedback:

Participants of the meeting were asked to provide feedback for each of the meeting’s sessions and an overall impression of the meeting. Twenty-three participants provided some feedback for the various sessions and 89 free text comments were contributed. The numbers of comments per session ranged from 3 comments for each of the sessions Global Update and Serology, to 9 comments for the Genotyping Updates. A total of 16 comments were provided for the overall impression of the meeting. The mean score of the feedback for the sessions was 82% and the overall meeting response was 87% (Table 1).

Table 1: Satisfaction Index

The comments provided by the participants who answered the feedback questionnaire could be summarised as generally being very positive and participants found the sessions to be informative and interesting with good discussions and everyone was given an opportunity to contribute. However, many reported that the meeting agenda was too packed; some of the presentations were too long and consequently there was insufficient time for more comprehensive discussions. One commenter said the Geneva venue was ideal and that they enjoyed the informal manner and opportunities for meeting colleagues, interacting with them and planning. Others suggested improved guidance on content of presentations to avoid repetition and that regional presentations were too long but individual country experiences were appreciated.
DRAFT RECOMMENDATIONS

LABORATORY DATA
1. Ensure completeness and timeliness of laboratory data at global level (WHO and Regional offices)
2. To move to case-based data reporting
3. Ensure compatibility of epidemiological and laboratory surveillance data
4. Genotyping paper on epidemic nature of genotypes

LABORATORY PERFORMANCE
5. Ensure all laboratories are enrolled in the annual WHO accreditation program, which includes proficiency testing (serology and molecular where applicable), confirmatory testing, and annual review (by correspondence or onsite).
6. A revised WHO accreditation checklist will be rolled out by end of 2013. Its design will aid in providing laboratory evidence for the verification of elimination.
7. The network laboratories should be using an in-house run control and record the outcome of the assay in a graph (according to Westgard rules). If laboratories do not have access to such run-controls, WHO can help in acquiring such samples. However, network laboratories are requested to retain samples and if possible share with network laboratories.
8. Serologic proficiency testing has become integrated component in WHO accreditation program. Laboratories should provide not only laboratory results but also include additional data in their report like (...)

CRS SURVEILLANCE
9. LabNet laboratories should participate in studies to assess challenges to implementing CRS surveillance (e.g. gaps in knowledge in laboratory persons regarding CRS surveillance (e.g. PPV of clinical diagnosis), specimen transport. A focus should be on countries planning RCV campaigns (GAVI countries).
10. LabNet laboratories, particularly in rubella endemic areas or where CRS is at high incidence, should try to participate in studies to develop new/improved techniques for CRS surveillance.

SEROPREVALENCE
11. LabNet laboratories are requested to support the WHO in developing guidance for best practices in seroprevalence studies/activities. Considerable expertise exists in the LabNet on such activities.
12. Initial support should be regarding laboratory testing, including (but not be limited to) assessing the performance of various IgG ELISA kits with attention to their use in seroprevalence studies rather than diagnostics, guidance on the interpretation of test results (e.g. use of qualitative versus quantitative measures, seropositivity vs. immunity, etc.), and possible utilization of reference serum(a). This support likely requires a temporary LabNet working group.
13. Many serosurveys are either ongoing or planned for the near future. Several of these studies involve a comparison of the ability to detect measles/rubella IgG in serum and oral fluid. LabNet should assist with protocol development and facilitate testing and data analysis so that the relative performance of the assays kits and protocols used can be monitored. LabNet should consider suggesting additional testing when possible to evaluate existing or new EIA kits and other serologic methods such as Luminex and rapid neutralization assays.

RUBELLA NOMENCLATURE AND DATABASE (RUBENS) AND MOLECULAR DETECTION
14. LabNet will support the new developments described in the upcoming WER and the initial meeting of the RubeNS steering committee.

15. LabNet laboratories should submit measles and rubella sequence information to MeaNS and RubeNS in a timely manner. NL directors should ensure that the lab has appropriate access to the databases and that staff members are properly trained to submit data. The genotype database at WHO/HQ will be phased out. LabNet laboratories should follow the recommendation of the MeaNS and RubeNS steering committees and make recommendations to the steering committee members for improving MeaNS and RubeNS.

16. Protocols for generating expanded sequence windows for measles and rubella should be submitted to the WHO SharePoint site.

17. Now that over 30 complete genomes of wild-type rubella viruses are available, additional conserved targets for primers and probes in real-time assays should be evaluated, seeking to decrease problems resulting from heterogeneity in binding sites in newly discovered wt-viruses. These evaluations should include clinical specimens, since performance will depend, in part, on the copy number of binding sites, i.e., on the proportion of genomic and subgenomic Rubella virus RNAs in clinical specimens.

18. LabNet supports the development of etraining materials to enhance training activities and facilitate, at least, refresher training and "between course staff changes". Specific support likely includes participating in development and evaluating training materials and facilitating the utilization of such materials (CDC).

19. PHE will be developing instruction video on oral fluid collection and processing to aid in the serological and molecular investigation of acute measles and rubella cases and seroprevalence studies.

20. The protocol for molecular EQA will be finalized by the beginning of the 3rd quarter of 2013. LabNet will initiate molecular EQA in 2013 with selected laboratories in each region to test the molecular PT panel and report by February 2014. Regional coordinators are requested to indicate maximum 5 laboratories in their region to participate in the first round.

21. WHO is committed to ensure high expertise in labnet on molecular diagnostics through individual training (CIV) and workshops (PAHO, SEARO, WPRO).

22. LabNet to evaluate the usefulness of extended window and full length genome sequencing as a tool for the WHO measles-rubella programme.

23. Laboratories and laboratory performance will be a critical part of the verification of elimination of measles, rubella and CRS. NLs, RRLs and RLCs should work closely with their regional verification commissions and monitor the laboratory performance indicators of each laboratory. The RLC should suggest that the national and regional verification committees contain at least one laboratory expert (should be laboratory expert without link to measles-rubella).

24. The WHO genotyping database will now be maintained by the MeaNS and RubeNS websites. Laboratory methods will be posted on the WHO SharePoint site. Submitters should make sure their protocols have a date or version control number so that the most recent versions of the protocols will be easily identified.

NEW TRAINING MATERIALS (ETRAINING)

20. LabNet will support the development of etraining materials to enhance training activities and facilitate, at least, refresher training and "between course staff changes". Specific support likely includes participating in development and evaluating training materials and facilitating the utilization of such materials (CDC).

MOLECULAR CAPACITY AND EQA

20. The protocol for molecular EQA will be finalized by the beginning of the 3rd quarter of 2013. LabNet will initiate molecular EQA in 2013 with selected laboratories in each region to test the molecular PT panel and report by February 2014. Regional coordinators are requested to indicate maximum 5 laboratories in their region to participate in the first round.

VERIFICATION OF ELIMINATION

23. Laboratories and laboratory performance will be a critical part of the verification of elimination of measles, rubella and CRS. NLs, RRLs and RLCs should work closely with their regional verification commissions and monitor the laboratory performance indicators of each laboratory. The RLC should suggest that the national and regional verification committees contain at least one laboratory expert (should be laboratory expert without link to measles-rubella).

WHO SHAREPOINT

24. The WHO genotyping database will now be maintained by the MeaNS and RubeNS websites. Laboratory methods will be posted on the WHO SharePoint site. Submitters should make sure their protocols have a date or version control number so that the most recent versions of the protocols will be easily identified.

ALTERNATIVE SAMPLING
25. Expand use of FTA cards for transporting clinical samples and viral isolates. GSLs and ERRLs should develop detailed protocols to share with RLCs.
**Meeting feedback form**

**Name (optional):**

Please provide an overall score for the usefulness of every session (1: not – 5: very) and a written comment.

<table>
<thead>
<tr>
<th>Session 1: Opening</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>7</td>
</tr>
</tbody>
</table>

Comment:
- Clear and precise
- Brief and precise and clearly gave objectives. Perfect
- Nice, replaced, welcoming format
- Can be left out except admin announcements

<table>
<thead>
<tr>
<th>Session 2: Global update</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

Comment:
- **Good summary**
- **Comprehensive**
- **Short overview of new development is useful**

<table>
<thead>
<tr>
<th>Session 3: Regional updates, progress and challenges</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>7</td>
</tr>
</tbody>
</table>

Comment:
- Important session but too long. Provide data up front and focus on discussion
- Good work in the field
- Best part were country presentations
- Too long
- Sub regional presentations appreciated, but shorter
- Too long
- Necessary
- Interesting but country presentations to be left out and included in regional updates
### Session 4: CRS surveillance

**Comment:**
- Useful
- Difficult to implement in the field
- Good start. Focus only on developing countries
- Informative
- Interesting as not yet implemented everywhere. Some info on problems and challenges in laboratory testing would be of benefit

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0</th>
<th>0</th>
<th>2</th>
<th>14</th>
<th>7</th>
</tr>
</thead>
</table>

### Session 5: Verification of elimination and the role of labnet

**Comment:**
- *expand in future based on developing experience*
- good presentations
- *more guidelines and indicators needed to monitor progress*
- Too late in the day, repetitive
- Too many presentations from too many regions; 2 is enough
- Overall overview useful. Shorter. Regions should focus on essentials/special. Many repetitions

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>9</th>
<th>5</th>
</tr>
</thead>
</table>

### Session 6a: Serology

**Comment:**
- session too long. Relevance for the labnet missing
- recommendation should be given on which kits to use
- Description of new techniques and developments that are of interest for the labnet audience

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>11</th>
<th>6</th>
</tr>
</thead>
</table>

### Session 6b: Serology/Performance

**Comment:**
- excellent presentations, esp standardization of rubella avidity
- very good start and to be sustained
- separate discussions on seroprevalence and case classification needed
- very nice and comprehensive analysis

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>12</th>
<th>6</th>
</tr>
</thead>
</table>

### Session 6c: Serology/Seroprevalence

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>0</th>
<th>2</th>
<th>2</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
</table>
**Comment:**
- need for standardization for rubella avidity testing clear
- excellent, to be taken forward
- Goodson presentation not relevant to audience
- Good discussion
- Especially the more general talks were quite interesting and useful for preparing guidelines for seroprevalence studies

**Session 6d: Serology/Rubella**

| 1 | 2 | 3 | 4 | 5 | 0 | 0 | 2 | 9 | 10 |

**Comment:**
- Fun, but no conclusion
- Good presentations
- Excellent discussions
- Excellent update and discussion
- Very interesting discussions and important problems were highlighted

**Session 7: Molecular methods, training and quality assurance**

| 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 7 | 8 |

**Comment:**
- Excellent presentations
- Excellent presentations and discussion
- Not enough time to discuss important issues

**Session 8: Regional breakout session**

| 1 | 2 | 3 | 4 | 5 | 0 | 0 | 3 | 8 | 8 |

**Comment:**
- Excellent, team building, needs more specific topics
- Good to identify priority areas and challenges
- Very well organized. Each region got chance to present future plans
- Good to discuss but focus should have been on challenges not achievements
- Plenary session too long and lacking focus
- Excellent new initiative
- Useful, feedback not. Better feedback needed

**Session 9: Measles genotyping updates**

| 1 | 2 | 3 | 4 | 5 | 0 | 3 | 3 | 9 | 5 |

**Comment:**
- Different approach needed, focus on vaccine introduction and elimination. Epidemiological context missing
- Shorten substantially with focus on ONLY genotyping. Repetition
- Excellent presentations on various genotypes
- Too lengthy
- Need to expand genotyping activity
- Missing epi linkage
- Important but too long
- Regional updates only
- MeaNS report now to take over making session redundant, shorten or omit. Regional genotyping overview could be included in regional updates

| Session 10: Genotyping rubella incl RubeNS | 1 2 3 4 5 | 0 1 3 12 3 |
| Comment: | | |
| - Needs link with rubella initiative in AFR |
| - Shorter |
| - Good presentations |
| - Evolving, but essential |
| - Nicely showed urgent need for data |

| Session 11: R&D | 1 2 3 4 5 | 0 0 7 9 3 |
| Comment: | | |
| - Valuable |
| - Good |
| - Innovative |
| - Confusing session |
| - Interesting |
| - Some interesting, and pity because of time |

| Overall impression of the meeting | 1 2 3 4 5 | 0 0 1 7 6 |
| Any other comments, suggestions: | | |
| - Agenda too condensed |
| - Improvement of chairs |
| - More time for discussion needed |
| - Presentations too long, not enough time, but exciting and useful. Everybody was given a chance to talk |
| - Fewer topics |
| - Very well structured and well organized. Encouragement to India welcome. Luminex promising, more laboratories to be involved |
| - Nice, too long, too many subjects. More comfortable next year |
• Nice, too much info, not enough time for discussion. LC should use less slides. Less presentations from RRL. Time lost for discussions
• Coherence missing, standardize feedback
• Should remain in Geneva as meeting venue was ideal. Coffee in the morning. Good opportunity to meet, interact and plan
• Agenda too long. Not enough time to discuss. Separate meeting from polio
• Agenda too packed, decrease # presentations, more time for discussions
• Jam-packed but informative
• Improve guidance on content of presentations to avoid repetition
• Less presentations and more time for discussion
• One presenter for slides from different people is good idea to deal with time constraints

Thank you for your feedback.