REPORT ON THE 12TH WHO GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK MEETING

David Featherstone

ISTANBUL, TURKEY
22-24 SEPTEMBER 2014
The Global Laboratory Coordinator Dr Mick Mulders welcomed participants to the meeting and presented the objectives and deliverables:

MEETING OBJECTIVES

1. Review and discuss current status and management of the Global Measles and Rubella Laboratory Network (GMRLN) to develop and strengthen technical capacity and structure of LabNet

2. To provide WHO staff and LabNet representatives with technical updates on laboratory-related issues to measles and rubella control and quality assurance

3. To determine how best to meet current and future challenges for the GMRLN

MEETING DELIVERABLES

Develop recommendations with implementation timelines to ensure a strong, fully functional and well-performing WHO Global Measles Rubella Laboratory Network

The meeting agenda was outlined and a brief summary of the recommendations from the 11th Global Measles and Rubella Laboratory Network 2013, was presented. The agenda was divided into 6 specific sessions as defined in the full agenda (Annex 1). Forty participants attended (Annex 2), including the 6 regional laboratory coordinators, and representatives of the global specialized and regional reference laboratories, selected national and sub-national laboratories and two consultants. One representative from the US CDC (Joe Icenogle) was unable to attend in person but fully participated through WebEx.

A summary of the presentations made are outlined below. All presentations are available in full on the following link:
https://www.dropbox.com/sh/3csnb7cuj41mbcm/AACMAxQNacUgAui4Xa7xi3la?dl=0
SESSION 2: GLOBAL UPDATES ON MEASLES AND RUBELLA ELIMINATION

AN UPDATE ON THE GLOBAL MEASLES AND RUBELLA PROGRAMME AND SURVEILLANCE

was made by Robert Perry, WHO, HQ. He reported that globally, MCV1 coverage has levelled off at 83-84% for the past 5 years. Three regions still have MCV1 coverage <90%, AFR, SEAR, and EMR. AMR, EUR and WPR have sustained MCV1 above 90% for at least the past 9 years. MCV2 coverage is substantially lower than MCV1 with even wider differences between the Regions. WPR and EUR report coverage >80% and the African Region with coverage <10%. However, there is a steady increase in MCV2 coverage overall with the global estimate reaching 53% in 2013.

In addition to routine delivery through routine services, 33 countries carried out campaigns with M or MR vaccines in 2013. Nearly 200 million children were reportedly vaccinated in these campaigns and over three quarters of all campaigns provided other vaccines in addition to measles vaccine, mainly rubella vaccine and OPV. However, 21 million infants missed MCV1 in 2013 with 60% of these living in India, Nigeria, Ethiopia, Indonesia, Pakistan and DRC.

There is a lack of integration of rubella with measles and there is a large inequity between coverage with RCV vaccine in The Americas, EUR and WPR, all with coverage >90%, and EMR, SEAR and AFR, with coverage <50%. The global estimate of RCV coverage, while increasing gradually, was only 43% in 2012.

It was reported that there is a tight supply situation for M and MR vaccines. One manufacturer has withdrawn from the market, however, Serum Institute of India, the supplier of M and MR for UNICEF, states they can meet the overall demand requirements for 2014 but there may be issues in the timing of supplies.

SURVEILLANCE

There was a sharp decline in measles cases following 2007 but a resurgence occurred in 2010 and 2011 but these levels have declined in 2012 and 2013. However, there are caveats due to some countries under reporting measles cases. For example, DRC reported 89,000 through the AFR aggregated reporting system but 800 were reported through the WHO/UNICEF JRF.

Field investigation of the 15 largest outbreaks globally in 2013 (figure 1) indicates that children are being missed both in routine immunization services and in SIAs. In many of these outbreaks coverage data did not reveal the gaps in immunity – it is only when the outbreaks occur that the programme weaknesses become evident.
IN SUMMARY

- Based on current trends and programme performance 2015 global targets will not be achieved on time

- There is a need to move surveillance to “elimination standard” quality, including:
  - Case-based data at all levels: more timely, critical analyses, quality indicators
  - Better handle large outbreaks or other high-incidence times
  - More in-depth case investigations
  - Increase rubella reporting and CRS too
  - Linkable surveillance and laboratory data

- Official incidence data is based on annual data collection (JRF). Currently there is an 18 months reporting lag
• RVC and NVCs provide encouragement and momentum towards achieving regional elimination goals

• There is a need to seize every opportunity for MR – “business as usual” will not be enough:
  – Ensure HCW immunized against measles and rubella
  – Change practices and policies to reduce missed opportunities and permit opening of vials for 1 child
  – Vaccinate with MCV1 even >12m of age
  – School entry screening

Questions: What do the delays in polio eradication impact on measles elimination and also Ebola outbreaks?

Answer: For Polio the challenges reflect those challenges of each country’s challenges. Can use the campaign to include measles and vice versa.

Nigeria had problems with measles campaign as couldn’t utilize the polio programme to assist. In Pakistan they were able to use the same

Many staff in AFRO have been pulled into Ebola work and only a skeleton staff are working on vaccination programmes. Measles campaigns in the infected areas have been delayed.

In countries with big outbreaks there are challenges in reporting cases through case based surveillance systems. Line listing all cases in big outbreaks is a big challenge and what advantages accrue?

Mick Mulders, WHO HQ Global Laboratory Coordinator, presented an

UPDATE OF THE GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK.

There has been an increase in number of laboratories to 713, primarily due to SEAR focusing on building its LabNet for measles surveillance now they have eradicated polio.

The LabNet has a critical role in the provision of laboratory indicators to meet criteria for verification of elimination. Recent highlights in the continual development of the LabNet include:

• Revised accreditation checklist with
• Accreditation procedures designed to reduce workload of the regional coordinators
• A molecular EQA program rolled out
• Genotyping databases, RubeNS has been operationalized (1075 entries) and MeaNS has been updated (19,000 entries)
• Seroprevalence guidelines are being developed with the support of the Bill and Melinda Gates Foundation (BMGF)
• Molecular methods workshops were held in SEAR and EMR
• A serology workshop was held in AFR
• Rubella IgG standardization of testing is being established

In 2013, the LabNet tested 320,359 suspected measles cases and 76,531 were laboratory confirmed. Up until August 2014, 242,935 suspected cases were tested by the LabNet and 72,477 were laboratory confirmed.

For rubella, in 2013, 100,861 specimens were tested with 10,489 found IgM positive. For 2014 (to August), 59,807 specimens were tested and 7,253 found IgM positive.

Sharing of genotype information with WHO from some countries has been a challenge. For the 12 months July 2013-June 2014, 150 Member States reported measles cases, 130 Member States reported laboratory data to WHO, 102 Member States reported laboratory confirmed cases, but only 48 reported genotype information. For rubella, from 95 Member States that reported laboratory confirmed rubella cases in 2013, only 6 reported genotypes to the genotype database.

Quality assurance has been strengthened with a molecular EQA developed by CDC and many laboratories have developed internal QA programmes. Diagnostic PCR kits have been developed and provided to those laboratories requesting them.

Some of the constraints and weaknesses of the LabNet include:

• Data timeliness and completeness,
• Establishing or enhancing case-based surveillance
• Private laboratories completeness of surveillance data
• Surveillance insufficiently recognized by donors as critical to the Measles and rubella initiative
• Genotype data reporting
• Increasing workload in coordination and testing
• Elimination goals now in all WHO Regions
• Introduction of rubella vaccination increases surveillance demands
• Need to perform additional laboratory tests in countries with low incidence of measles and/or rubella

Activities proposed to strengthen Laboratory based surveillance include:

• Increasing country ownership and investment in network laboratories, including molecular capacity
• Global migration to weekly case-based surveillance and reporting, and integrating epi and laboratory
• Enhancing genotype surveillance (including rubella)
• Strengthening laboratory capacity and building the network
• Introducing case-based surveillance with laboratory confirmation in SEAR
• Strengthening coordination of WHO laboratory networks with increasing workload
• Securing sufficient funding and staffing
• Development of global guidelines to conduct measles and rubella serosurveys

Operational planning for 2014-2015 for the regional coordinator will involve on-site accreditation visits to: CAN, PAK, MAL, TOG, CIV, INO, CAV, IND, UK, LUX, DPRK, THA and participation in meetings, provisionally planned for:

• SEAR (27-31 Oct)
Scientific conferences

ESCV (28 Sep-1 Oct)

ASLM (30 Nov-4 Dec)

Workshops:

Molecular workshop EUR, AFR (combined in Tunis?)

Training (CIV)

A link to the global coordinator’s planning calendar is available on

(removed for privacy reasons)

Questions:

Annick: Due to the current Ebola outbreak the annual laboratory directors meeting is postponed to 2015

Marilda: Data for Chile is not complete.

Mick: This will be corrected in the next month’s update of data.

EURO REPORT

Myriam Ben Mamou, Laboratory Coordinator, EUR, provided an update on the European region.

The European region has seen some resurgence in measles and rubella cases since the lowest reported incidence rates which were recorded in 2009. Most of European suspected measles cases are either laboratory confirmed or epi-linked.

In 2013, Georgia had a large measles outbreak with 7830 cases reported, although most were clinically compatible. Turkey reported more than 7000 laboratory confirmed cases. The measles outbreak in Ukraine continued into 2014. The Netherlands reported 2499 cases in 2013. Georgia, Russian Federation, Bosnia Herzegovina, Ukraine and Italy were the countries reporting the most cases in the region in 2014.

Healthcare settings have been identified as the source of outbreaks in a number of countries with unprotected healthcare workers responsible for disseminating cases. Educational facilities were also found to be settings for the spread of infection.

In 2013-14, genotypes B3, D4 and D8 predominated with D8 genotype the most common reported in 2013, and B3 the most common in 2014.
Rubella infections in Europe have seen a resurgence since the low of 9464 cases reported in 2011. In 2013, 38585 were reported in Poland with most cases clinically confirmed. In 2014, Poland reported 4501 cases up to July. Very few rubella genotypes have been identified in Europe in 2013-14, with just 2B reported from the UK.

Verification of measles and rubella in the region continues with 47 countries submitting annual status reports since 2010 and 16 countries are considered to have interrupted measles and 19 countries are considered to have interrupted rubella.

The European LabNet consists of 72 laboratories and more than 60000 specimens were received in 2013. More than 90% of these were reported within 7 days of receipt in the laboratory, however, only 29 laboratories are reporting more than 80% of results within 4 days. Accreditation performance is good.

Some of the challenges identified for the region’s LabNet are: data quality and timeliness of reporting, identifying measles genotypes in chains of transmission, procurement and shipment issues in Eastern Europe and financial sustainability of some laboratories.

The head of the Turkey National Laboratory, Gulay Korukluoglu, reported the activities of the National Laboratory (NL) and sub-NLs in Turkey.

The NL is situated in Ankara and 7 sub-national laboratories (SNLs) have been established. All test results are reported to the appropriate databases, LDMS, CISID and MeaNS, although there are some timeliness issues in reporting genotype data.

Up until 2006 Turkey had good control of measles but in 2011 cases were detected in Istanbul that spread to many provinces. Case numbers peaked in 2013, with 8042 measles confirmed by LabNet from 32640 cases tested.

In 2014, 787 positive cases were detected from 6679 cases tested. The SNLs in the country provide a good support for measles surveillance.

The main areas of the country affected in 2012-13 were the NW (Istanbul) and southeast provinces that were seeded from refugees from Syria and Iraq.

Age distribution of cases identified determined that most cases were under 9 years age of which half were under 4 years and 29% under 11 months. The vaccination status of cases identified showed that 41% were unvaccinated, 30% unknown, 16% with 1 dose and 2.5% with 2 doses. Current schedule is 1st MCV dose at 12 months and 2nd at 6 years. A study of mothers (N=398) and newborns identified that 20% of mothers and 40% of babies were measles seronegative.

Dr Kevin Brown of the Virus Reference Department, Public Health England, UK provided an update of the PHE Global Specialised Laboratory (GSL).

Clintech Company now produces MicroImmune assays under licence. Since the change there has been some ongoing problems with stability of the rubella antigen which have affected rubella IgM testing.
In 2013, the UK saw a surge of measles cases in Wales and England. The 11-20 year old age group predominated which is most likely due to the “Wakefield effect” when immunisation levels dropped after misleading reports of a linkage with MMR and autism 20 years ago. However, immunisation levels are now back on target.

In response to the outbreak in 2013, Wales established special vaccination clinics in hospitals/GPs in affected areas and also implemented school-based vaccination. In England, a catch-up campaign was implemented from April to September 2013 with the net result that cases reduced dramatically in both England and Wales.

Genotype D8 predominated in the outbreak but multiple other genotypes were detected. In 2014, a small number of cases were detected and genotypes were indicative of multiple importations with many having epi-links which indicated importation.

Rubella continues to be detected in very small numbers with 13 cases identified in 2013. One case of CRS was detected in 2014 after the infection of a UK resident during travel in Africa.

The problems due to the MicroImmune rubella IgM assay has meant that most rubella samples also tested by PCR for added specificity.

Judith Hubschen provided an update from the Regional Reference laboratory in Luxembourg.

For the measles proficiency test (PT), 27 laboratories participated and all gained 100% scores. For the rubella PT, 26 laboratories participated and 23 returned 100% scores with the remaining 3 scoring 95%.

For confirmatory testing, 17/18 laboratories that participated achieved a 100% concordance for measles and for 1 laboratory, less than 90% concordance was found. For rubella, 18/19 laboratories achieved 100% concordance and 1 laboratory got an 86.6% score.

Some of the challenges reported for the sub-region were: suboptimal communication from the NLs to the RRL requiring several reminders to send samples for confirmatory testing, leakage of samples during shipment, large numbers of negatives selected, Siemens rubella IgM kit reported to be prone to false positive results.

For MeV genotyping: 71 sequences were received from Georgia (D8), 14 sequences from Turkey (D8, B3), 8 sequences from TFYR Macedonia (D8), 6 sequences from Senegal (B3), 6 sequences from Bulgaria (D8), 5 sequences from Syria (D8), 3 sequences from Bosnia and Herzegovina (D8), 2 sequences from Luxembourg (B3).

Some of the sequencing challenges identified were: lack of appropriate specimens for rubella genotyping, specimens other than serum/blood often not available for measles and basic information missing for WHO name composition.

Other RRL activities included distribution of laboratory reagents, panels and protocols to M/R laboratories and training of M/R laboratory staff in Senegal, and Bulgaria. A postgraduate student from Gabrichevsky Institute conducted research within his PhD program.
Research activities related to Measles and rubella carried out by the Luxembourg RRL included: outbreak investigations, seroprevalence studies, rash/fever disease surveillance, next generation sequencing technology for measles complete genomes, and vaccination campaign monitoring.

Annette Mankertz provided an update on the Robert Koch Institute (RKI) RRL, Berlin, Germany.

It was reported that 2014 was a quiet year for measles in Germany and RKI has utilised the spare time to produce multiple publications of recent data.

One of the challenges of measles surveillance in Germany is the number of private laboratories in the country performing measles tests. RKI contacted more than 100 private laboratories in the country and asked them to share data with RKI. However, it was found that in most cases, epi data is missing and serum IgM tests for acute cases cannot be distinguished from tests completed for other reasons e.g. Maternal screening.

It was reported that countries under the responsibility of RKI have been encouraged to ship samples for molecular testing on FTA cards.

Plans for a second phase of the KiGGS study was announced for 2014-2016. It will be a cross sectional plus longitudinal study of 25000 children 1-17yrs old to identify risk factors for being unprotected and comparing seroprotection with vaccination history.

Sergei Shulga presented an update of the progress the EURO RRL in Moscow covering the NIS region from 2013-2014.

Big measles outbreaks in Georgia, Ukraine and the Russian Federation were reported in 2013 and 2014. The predominant genotypes detected were D8 (Frankfurt) in Georgia, D4 (Manchester) in Ukraine and 5 lineages of D8 genotype in the Russian Federation. Measles strains in Georgia and Ukraine demonstrate very low diversity confirming fast spread of infection into susceptible populations following importation of the virus. Prolonged transmission of measles in Ukraine could be also sustained by repeated importation of D4 (Manchester) lineages from other countries of the EURO WHO region where this lineage is widely distributed. Moreover recent data demonstrate independent importation of two different lineages of D8 genotype into Ukraine.

The Russian Federation has reported many outbreaks over past 4 years with a predominance of cases from Moscow, the Moscow region and Southern provinces of the country. However genotyping data demonstrate much higher diversity of circulating viruses and shift of the predominant genotypes. Virus lineage D4 (Bandarabas) had been predominant between 2011 and 2013 but as its transmission was interrupted in the beginning of 2013 strains of D8 genotype became predominant. D8 genotype strains isolated in Russia presented by two "main" lineages (Frankfurt and Willupuram) and several more groups of viruses. Furthermore few outbreaks were linked to limited local transmission of D4 (Manchester) lineage and some lineages of B3 genotype. On the whole despite relatively high incidence and prolonged transmission of virus that are shown for some regions the majority of the territories reported low incidence or local outbreaks followed importation of infection from different countries over the world.

Proficiency testing of all 24 laboratories in the sub-region saw 100% score gained for both measles and rubella. Except for two laboratories (Azerbaijan and Ukraine) that demonstrated concordance of
94.4% and 96.7% respectively for measles IgM confirmatory testing all the rest achieved 100% concordance for confirmatory testing for both measles and rubella. Locally produced kits, Vector Best for measles and Ekolab for rubella are used in the Russian Federation. Other NIS laboratories use Siemens that are provided by WHO. The RRL uses Siemens only for confirmatory tests for NIS.

The regional laboratory coordinator, for the Western Pacific Region, Youngmee Jee, provided a summary of the region’s progress towards measles elimination and rubella control.

She reported 94% reduction in measles cases from 2000 to 2012 in the region and 84% reduction in estimated measles deaths. It is likely that 34 of 37 countries and areas have interrupted transmission of endemic measles virus. In March 2014, the Regional Verification Commission verified measles elimination for 4 Member States: Australia, Macao (China), Mongolia, and the Republic of Korea. However the region has also seen a relative resurgence of measles in 2013-2014.

China has seen a measles resurgence with 26912 cases reported in 2013 and 42194 in 2014 (as of August). There have been large outbreaks in Philippines (16180 cases confirmed), PNG (2153 confirmed) Viet Nam (3688 confirmed), New Zealand (253 cases confirmed) and an outbreak in the Federated States of Micronesia (420 reported).

Supplementary immunisation campaigns have been carried out or are planned in the next 12 months for most of the countries with outbreaks.

Five countries have not introduced 2nd dose measles and rubella but Lao, PNG, Solomon Islands and Viet Nam have plans for introduction.

Some of the challenges identified for the region include: the widespread outbreaks pose a risk of the region losing previous gains, some countries are reporting shortage of kits.

However, the WPR LabNet is performing well with 51/52 network laboratories accredited by WHO as of September 2014. Improved molecular surveillance has occurred after capacity strengthening through three workshops held at the HK (China) RRL. All laboratories passed the global PT for measles and rubella and regular confirmatory testing is performed with >90% concordance achieved in 2013.

Genotype information from the region shows evidence of B3 (Harare) in most countries reporting measles and the subsequent spread of B3 to multiple countries regionally and globally from the Philippines outbreak. Viet Nam reported D8 and H1, as well as B3. Six countries in the region reported rubella genotypes in 2013, Mongolia, Hong Kong, China, Japan, Philippines and Brunei.

Plans for 2014-2015 include the accreditation of all countries but with priority laboratories undergoing an onsite visit and others assessed by a paper-based review, and the strengthening of strain surveillance of both sporadic and outbreak cases.

Sadly, Dr Jee announced her probable from the LabNet with effect sometime this year.

An update of the GSL Activities for Measles and Rubella in Japan was provided by Dr Makoto Takeda, of NIID, Tokyo, Japan.

It was reported that with the support of JICA, NIID held a training course in the Laboratory Diagnosis Techniques for the Control of Vaccine Preventable Diseases, including Poliomyelitis and Measles.

NIID supported Lao PDR in investigating the seroepidemiology of measles and rubella among the general population in a nationwide multistage cluster sampling of 2,184 participants. Dried blood spots (DBS) were used and tested for measles and rubella at NIID by IgG ELISA.

NIID also held a research meeting with selected Prefecture Laboratories in Japan on the development of real-time PCR standard protocols for measles and rubella in Japan, investigation of the quality control of molecular surveillance of measles and rubella in Japan, improvement of virus detection techniques, and development of an easy and affordable multiplex assay kit for rash and fever diseases.

Janice Lo reported on the activities of the Hong Kong RRL, Public Health Laboratory Services Branch, Centre for Health Protection, Hong Kong SAR, China

Confirmed measles and rubella cases in the city are low with 38 measles cases detected in 2013 and 43 in 2014 (as of 31 July). About half were import related in 2013 and about a third in 2014. For rubella, 11 cases were confirmed in 2013 and 6 in 2014.

Nosocomial measles cases have been detected where most are detected in individuals which are under the minimum vaccination-age. Zero dose or unknown vaccination history make up those affected over 20 years of age, which are almost half of all cases reported.

Laboratory performance is good with reporting within 4 days for more than 90% of tests performed.

Maintaining the capacity for virus isolation in the laboratory has allowed for a proportion of cases to have virus detected, with 40 isolates found in 2013 and 2014. Measles genotype H1 predominates, mostly from Mainland China, but also B3, D8 and D9 genotypes were detected. Most of the B3 identified had epi-links to the Philippines’ outbreak but not all. One of the three D8 genotypes found had epi-links to India. Rubella genotypes detected were 1E and 2B in 2013 and 2B in 2014.

Seroprevalence of measles continues, using EIA, and if negative followed by PRNT, but it is likely that Luminex testing using multiple antigens will be introduced with the support of RIVM.

The RRL activities for the region saw all countries supported by the RRL having >90% concordance for confirmatory testing results. The laboratory hosted the measles and poliovirus culture training workshop in May 2014 with 17 participants from 8 WPR countries attending.

An update from the China CDC RRL was provided by Dr Xu Wenbo.

After the national measles campaign in China in 2010, 100 million children were vaccinated and a marked reduction of cases followed. However, a resurgence of measles cases occurred in 2012 and continued into 2013 and 2014.

In 2014, most cases were detected in the eastern provinces but also several foci of cases were found in the western province of Xinjiang, close to the border with Pakistan. Age distribution of cases saw a high percentage under 1 year with a peak at 4-10 months and zero dose. Some provinces have more
than 60% cases in >20 year olds, especially in the more developed provinces, most with unknown vaccination status reported.

Surveillance performance indicators in 2013 are all meeting targets.

Serological tests performed by the China LabNet totalled 93558 for measles and 87739 for rubella in 2014 (to July), with 44.8% positive for measles and 2.7% positive for rubella. In 2014, 44% cases with samples are IgM positive

The virus detection workload increased in 2013-2014, with a total of 3038 sequences obtained in the 12 months July 2013 to June 2014, and confirmed genotypes: 2950 H1 (97%), 19 D8 (0.6%), 38 D9 (1.3%), 9 B3 (0.3%) and 1 G3 (0.03%).

The quality of the China LabNet continues to be strong with all laboratories passing the annual global PT and confirmatory results are 29/30 100% concordant with one laboratory (Tibet) receiving no samples. Annual accreditation reviews continue to show high performance. Training workshops are held every 2 years for provincial laboratories staff and are necessary due to the continual turnover of laboratory staff.

China continues to face challenges in the elimination of measles and have raised concerns that adult cases are due to secondary vaccine failure. The China LabNet is concerned that indirect IgM has a lower sensitivity for detecting secondary vaccine failure and is considering using rtRT PCR to confirm cases.

A review of **Measles in Beijing Province** was presented by Xu Wenbo in collaboration with Fang Huang.

More than 2222 measles cases (10.8/100,000) were reported in 2014, (Jan-Jul), a 3.7 fold increase compared with the same time in 2013 and almost equivalent to the number detected in 2010, just prior to the national campaign. Age and vaccine status of cases is similar to national data.

A breakdown of the residency of measles cases in Beijing shows a high proportion of cases in the floating population (adults and children) especially after the spring festival when people return from visiting their homes in other provinces.

Beijing province had 1021 measles sequences detected in 2014, compared to 367 in 2013. H1 genotype predominated but an outbreak of D8 in adults occurred with 35 sequences found in 2013.

The Beijing Provincial laboratory performed a comparison of sensitivity of rt RT PCR and IgM in confirming cases. Of 443 cases, it was found that 33.6% would be missed with IgM alone, especially with samples collected in the first 3 days after onset. Almost 10% would be missed if only PCR was used. Good concordance between PCR and IgM was found for samples collected 4-5 days after rash onset.

A presentation on the **Measles and Rubella Laboratory Network Progress and Challenges in the Eastern Mediterranean Region 2014** was made by the regional laboratory coordinator, Hinda Ahmed.
As a result of half the countries in EMR being in conflict, measles elimination activities have been adversely affected. It was decided by member states that the 2015 target for regional Measles elimination will not be met and the global goal of 2020 was determined as the new elimination goal.

Large measles outbreaks have occurred in Lebanon and Syria, Sudan, Pakistan, and Somalia. Iraq Iran, Syria and Jordan have increased measles incidence following years of low incidence. Syria had very high coverage but since the conflict started, routine immunization has suffered resulting in outbreaks. D8 genotype was identified from cases.

The Lebanon outbreak has reached an incidence of 407/100,000 in 2013. Syrian refugees are moving into the community from border camps and spreading measles virus.

All countries apart from Palestine have reported baseline genotype information. Palestine detected only one case of measles in the past 4 years but were unable to collect any genotype information from that case. B3 genotype predominated in the region.

Reporting timeliness within 4 days is excellent with all laboratories meeting the goal of 80%. All laboratories passed the global PT except Djibouti, which did not participate. A total of 21 of the 22 National Measles and Rubella Laboratories were accredited in 2013. Only Djibouti has not been accredited. Paper based accreditation has been challenging as laboratories have not responded in a timely manner and often the form is not adequately completed requiring back and forth communication.

Key issues to be addressed in the region include: timely reporting of data to WHO, timeliness and completeness of reporting genotyping data to MeaNS & RubeNS, two countries are reluctant to report genotype data, and rubella genotyping is weak. The Regional Office is working to improve reporting.


Dr Henda Triki provided an update of the Tunisia RRL for EMR. The RRL is responsible for 15 of the 22 countries in the region and received samples for validation from seven countries in 2013 and three in 2014. Concordance results were greater than 95% for both measles and rubella.

Eight countries in 2013 and five countries in 2014 sent samples for virus detection, to the RRL. Measles genotype B3 was detected in Sudan, Jordan, Libya, Somalia and Tunisia and D8 strains with identical sequences were found in Syria, Lebanon, Iraq, and Kuwait that were different from sequences found previously. Rubella 2B was found in Tunisia and Sudan.

A report from the Oman RRL was provided by Said Al Baqlani.

Oman RRL supports 9 EMR countries for Measles and Rubella IgM validation. Overall, countries achieved >99% IgM concordance with the RRL, however, not all countries report sample ODs making it difficult to gain maximum benefit based on qualitative results only.

In Oman, measles was confirmed in 46 cases in 2013 and 30 cases in 2014 (Sept), and rubella cases were laboratory confirmed in 16 cases in 2013 and 14 cases in 2014 (Sept).
Under the guidance of CDC-Atlanta the RRL plans to improve genotyping of Rubella virus.

The **South East Asian Regional update** was provided by the regional laboratory coordinator, Sirima Pattamadilok.

Following the certification of polio eradication in the region, all member states resolved at the 66th Meeting of the SEAR Regional Committee in September 2013 to adopt the goal of measles elimination and rubella/CRS control in the Region by 2020.

Activities conducted after adopting a measles elimination goal included: developing a regional case-based, laboratory supported measles/rubella surveillance started from January 2014. In support of this an Inter-country training workshop on Molecular technique was held in February 2014 and EPI data managers underwent orientation.

The 5th ITAG meeting, in 2014, made the following laboratory specific recommendations:

- Laboratories should be scaled up to be fully functional to meet the demands of greater number of tests and with a turnaround time within 4 days.
- Timor-Leste should enhance its current laboratory to “proficient” status in order to support case-based surveillance.
- Laboratory capacity should be enhanced to provide the genotype data for measles and rubella required to identify indigenous transmission, sources of infection and imported and import-related cases.
- By the end of 2015, all countries will have adequate access to an accredited national and reference laboratory (ies).
- Case-based surveillance for measles and rubella will be fully operational in all countries except for India and Indonesia which will be expanding case-based surveillance,
- All member states should share genotype information in timely fashion.
- WHO SEAR should provide a training workshop on laboratory aspects of CRS in 2015.
- By 2016, for verifying interruption of indigenous transmission and to identify imported and import-related cases, measles virus genotypes should be characterized in at least 80% of chains of transmission.

The current status of the SEAR MR LabNet is 37 laboratories and it is proposed that 43 will have been established by 2020. The number of specimens tested by SEAR laboratories peaked at 18000 in 2011, between 2009-2013, but once case based surveillance starts the numbers are expected to increase considerably.

The 2013 global PT results for the region are good and timeliness of reporting meets the requirement within 7 days except for DPR Korea and Sri Lanka, however Timor Leste has not responded. Timely and comprehensive genotype reporting needs to be improved.

In SEAR, currently 25 laboratories are WHO accredited and 11 have established a WHO approved quality assurance programme and 1 laboratory is pending accreditation.

The challenge for the region is for each country to establish a genotype database to identify endemic circulating and imported viruses.

An **update on the RRL at the National Institute of Health in Thailand** was presented by Atchariya Lukebua.
National Laboratory activities of the RRL include coordinating the 13 SNLs established in Thailand. In 2013, 920 cases were tested with 38% positive for measles and more than 93% reported within 4 days of receipt. PT results were 100% for 12 of the 13 laboratories and one achieving 95%. Confirmatory test results were all 100% except for 2 laboratories which have not shipped samples.

The RRL is responsible for 8 countries in the region and recent support activities include training for staff from Nepal and Timor-Leste laboratories in January 2014 and molecular testing in February 2014. Countries sending samples for confirmatory testing totalled 6 in 2013 and 5 in 2014. Indonesia has not sent confirmatory samples since 2007. Chennai laboratory sent dried serum to the RRL by post and comparative sera by cold chain, with concordant results. Bangladesh also sends dried serum samples. Concordance with the RRL’s results for all countries is generally high.

The RRL conducted a small study of four EIA kits (Siemens, Human Euroimmun and MicroImmune. Using Siemens as the “gold” standard, sensitivity results were: 97.25%, 89.9% and 100% respectively. Specificity results were: 96%, 100% and 87.8% respectively. Human and Euroimmun were cheaper than Siemens tests, based on the purchase price in Thailand.

The RRL also assessed the impact of storage temperature on the stability of dried serum spots (DSS). A total of 129 samples: Negative = 21 samples, Equivocal = 5 samples and Positive = 103 samples with OD values >0.2.

IgM Siemens kit was used for testing and Whatman 903 filter paper for the DSS. Extraction and testing followed the LabNet protocol

Storage of DSS at temperatures from 4°C to 45°C for periods of 3-30 days. Results showed good (>85%) concordance for up to 14 days at 4°C and for 3 days at room temperature. At 37°C for 3 days, 39% were concordant and at 45°C for 3 days 28% were concordant.

Lucky Sangal, Laboratory focal point for the WHO Country Office, India, presented *strengthening the measles and rubella laboratory network in India.*

Currently India has aggregated surveillance based on outbreak investigation and is not yet case based. A WHO-NPSP supported surveillance system is planned based on the AFP surveillance system platform, backed by laboratory support. This is currently covering 29 states with ~ 97 % of India’s population. Laboratory supported measles outbreak surveillance will be initiated in a phased manner with nation-wide surveillance established by the end of 2014. The reporting network will consist of > 40,000 reporting sites using public and private hospitals and different health care set ups, including traditional healers.

Using weekly reporting, 37,000 suspected cases were reported in 28 states in 2014, despite weak surveillance. Once case based surveillance is established, the number of cases is expected to increase exponentially.

Currently 11 Laboratories have been established with 2 more proposed. In 2013, 2061 samples were tested and 3511 in 2014 (Sept). In the 12 months, Sept 2013 – Sept 2014, 3 laboratories reported 80% of samples to the programme within the required 4 days of receiving samples, although 8 laboratories had completed testing within 4 days. Serum confirmatory test results for all 11 laboratories was 100% for measles and 8/11 met the >90% concordance requirement for rubella.
For molecular surveillance, efforts for collection of throat swab/urine samples were intensified in Sept 2013. In 2014, a total of 172 specimens were collected from different geographical areas and genotype information found for 34 samples.

As the LabNet in India undergoes expansion, consideration for using the Virus Diagnostic Laboratories of ICMR can be made although they will need kits and reagents. It is considered that at least one laboratory per state will be established.

**An update on the African Region** was made by the Regional Laboratory coordinators Drs Annick Dosseh and Charles Byabamazima.

The African Regional Goal is to achieve measles elimination by 2020. However, there is a stagnation of MCV1 coverage with regional coverage still below 80% and only 15 countries achieving 90% in 2013.

A combined laboratory network has been established for measles and YF in the region. There is one measles laboratory per country except for Nigeria which has four laboratories and Ethiopia, three. Due to their small size and good measles control, Seychelles, Mauritius and Cape Verde have not established measles laboratories.

The current outbreak of Ebola in West Africa has impacted on AFRO staff distribution in the region and the LabNet meeting in 2014 was postponed due to the outbreak. It does not appear that Ebola has affected the collection of measles samples in the affected countries, so far, when comparing an equivalent period in 2013. However, quarterly confirmatory testing has been impacted by refusal of DHL to ship any samples due to a fear of Ebola infection in the samples.

In the region, a total of 43782 specimens were tested in 2013 and 26956 in 2014 (until August). Most laboratories were reporting data within 7 days in 2013. However, in 2014, fewer than 50% Laboratories are reporting within 7 days as a result of funding delays in the first half of 2014 which caused a shortage of kits and impacted reporting timeliness. A total of 19 laboratories were affected and delays in detecting outbreaks by up to 120 days occurred.

The 2013 global PT was performed by 36 of 40 laboratories and all passed for measles and rubella.

Cote d’Ivoire (CIV) has received a large number of samples for confirmatory testing compared with the other RRLs. More than 2000 were received in 2013 and the shortage of kits has impacted their testing too.

Genotype information for 9 countries during 2013-2014 identified only B3 genotype.

On site accreditation visits are conducted each 3 to 4 years, however, Guinea Bissau, Liberia, Sierra Leone and South Sudan have never received an accreditation visit.

Planned activities for the region include: sequencing training at CDC for 2 CIV RRL staff (8 October-8 November 2014), Molecular/PCR training in 2015, On site serology training for 3 laboratories, Establishing measles laboratories in the remaining 3 countries without laboratories, Completion of measles laboratory accreditation (2014-2015), Consideration of full implementation of Oral fluid collection in selected countries.
Dr Herve Kadjo presented the recent **activities of the Cote D’Ivoire RRL.**

The CIV laboratory supports 5 NLs in the West and Central African region: Angola, Guinea, Liberia, Sierra Leone and Benin. The RRL has real-time and conventional PCR equipment and an ABI 3500 sequencer but sequencing activities will start after training is completed at CDC later in 2014.

The RRL received 2691 serum samples in 2013 and 1964 in 2014 (Sept) with 289 and 41 throat swabs each year, respectively.

For confirmatory testing, 634 samples were tested in 2013 and 964 in 2014. The increase in 2014 was due to a shortage of kits in some of the countries supported. All 17 laboratories which send samples except one achieved >90% concordance results for measles and all achieved >90% concordance for rubella.

Currently samples for molecular surveillance are sent to CDC but 2 CIV staff will visit CDC for molecular training and hopefully establish sequencing at CIV.

A small comparative study of OF and serum was undertaken and of 71 paired samples, 96% were concordant with the Siemens serum IgM.

The **Measles RRL and National Activities at the Uganda Virus Research Institute**, Uganda were reported by Barnabas Bakamutumaho.

UVRI supports NLs from Eastern Africa sub-region: Burundi, Comoros, Eritrea, Ethiopia, Kenya, Rwanda, Tanzania & Uganda and provides them with quality assurance and virus detection assistance.

Confirmatory testing shows consistently good performance of the designated NLs, however, Rwanda and Tanzania shipped no samples in 2013, but did so in 2014. Both countries showed slightly less than 90% concordance (89% and 87%).

Genotyping activities in Uganda identified measles B3 in border areas with DRC Tanzania and Sudan, and Kampala between 2011 and 2014. Rubella 1E was identified near border with Sudan and 1G was found over widespread areas of Uganda. Ten 1G sequences identified have yet to be reported to RubeNS.

Prior to the introduction of rubella containing vaccine, CRS surveillance has been conducted in 2 sentinel sites (Kampala & Entebbe) to provide estimated disease burden of CRS in Uganda.

Sheilagh Smit reported the activities of the **RRL for the African Region at NICD, South Africa.**

Recent changes to measles and rubella surveillance in South Africa have resulted in routine measles virus isolation being stopped and rubella virus isolation has never been implemented. Rubella IgM testing of specimens collected for measles surveillance stopped in March 2013 but may be re-established once rubella vaccination begins in 2015. Measles virus genotyping has been discontinued except in the event of an outbreak.

In 2013 NICD tested 8000 suspected measles specimens from national surveillance with 10 IgM positive and 36 equivocal. In 2014 (July), 4000 were tested, with 7 IgM positive and 10 equivocal samples found.
For the regional reference laboratory activities, due to delays in funding support from WHO, all regional measles genotyping activities stopped towards end of 2011. Confirmatory serology testing was allowed to continue because WHO provides the Siemens kits for this purpose. However, WHO funding has now been received and RT-PCR and genotyping activities have recently resumed.

South African measles IgM positive or equivocal specimens from 2013/2014 were tested under the umbrella of the RRL activities for genotype information. In 2013 and 2014, the nine NLs supported by NICD which sent samples for confirmatory testing achieved >98% concordance for both years. Angola now sends samples to CIV rather than NICD due to transport cost implications.

Sera sent by 9 NLs in the sub-region supported by NICD for confirmatory IgM testing found positive or equivocal were also tested by RT-PCR/sequencing.

Not all positive PCR samples were sequenced but all that were, were B3 and most were identical to B3 Harare 2009.

One cluster of B3 viruses from Namibia were found close to border of Angola and were unique to NICD’s database of B3s although they were not compared with the full MeaNS database.

Some of the challenges for NICD’s NL activities include: delays in specimen transport as more than 40% of specimens take longer than 3 days to reach NICD,

60-70% of specimens have Case Investigation Forms (CIF) but generally the required fields are not filled in. Data management is a persistent problem with data mining system used at NICD which misses cases and its format is not compatible with WHO’s database. NICD has plans to use Excel to overcome these issues.

Challenges reported for RRL activities, include: Samples for confirmatory testing are not sent within the stipulated time, Some NLs send samples sporadically, samples are sent without the electronic database or incomplete and/or incorrect data, national laboratories do not respond when asked to retest discordant samples and difficult to obtain epidemiologic info from NL or AFR databases.

The regional laboratory coordinator, Gloria Rey-Benito, presented an update on the Region of Americas.

There has been a resurgence of measles cases in the past 2 years following the interruption of endemic transmission in the Americas. In 2013, 473 cases were reported from outbreaks in Brazil, Canada and USA and numbers of cases from these countries reached a total of 1465 by August 2014. Confirmed rubella cases reported in the region have not been above 20 per year over the last 6 years. In 2014 (August), 4 cases have been reported, all related to importation.

Measles and Rubella surveillance indicators in the Region of the Americas, 2010 to 2014, show that the proportion of laboratory results reported in 4 days or under dropped to 69% from >85% in the previous 4 years. PAHO is working with the laboratories to improve this indicator.

Rubella
Members of the International Expert Committee (IEC) met in April 2014 with representatives of 30 countries in Paraguay, to review evidence that support the interruption of endemic measles and rubella virus in the Americas. The region has a goal of 95% coverage in all municipalities. Active searches were implemented in high risk areas in 16 countries with national commissions focusing on silent municipalities, those with high influx of tourists, migrants or displaced people, border areas and those with low vaccination coverage.

Retrospective search for CRS cases were implemented by 16 countries with national commissions in institutions.

The Americas LabNet consists of 165 laboratories with 141 SNLs: Argentina (21), Brazil (27), Canada (26), Colombia (11), Mexico (31) and Venezuela (25). All national and reference laboratories passed the global proficiency test except for one. The accreditation status of the MR National Laboratories as of 2014 is: 18 fully accredited and 6 provisionally. Canada, Dominican Republic and Honduras are planned for review in 2015.

Genotype information from the region indicated a pattern of multiple importations. Measles B3 and D8 genotypes predominated but also D3, D4, D6 (SSPE), D7, D9, and H1 were detected. Rubella 2B and 1E in 2013 and 2B and 1J in 2014.

In summary, the region of the Americas has used molecular epidemiologic data to document the lack of any measles endemic genotype circulating between 2002 and 2013 and no rubella endemic genotype was found between 2009 and 2013. The pattern of genotypes varying year-to-year and country-to-country is consistent with importation of measles and rubella. One country has sustained transmission of measles.

Some of the challenges the region is facing are: the need to improve the collection of adequate specimens for virological testing, frequent importation of virus from endemic areas in the world, unimmunized or under-immunized individuals, cases of primary and secondary vaccine failure, limited reliability of IgM assays mean that some cases may need to use a variety of serologic and molecular tests to aid classification, vaccine reactions are being confused with disease requiring the need for a more rapid and specific assay for detection, need to improve real time communication between epi and laboratory.

A review of **Measles in the USA**: 2014 was presented by Paul Rota of the global specialised laboratory, US CDC, Atlanta, USA.

Endemic measles was eliminated in the USA in 1994 and the country was declared free of endemic measles in 2000 and underwent verification in 2012. All cases subsequently are the result of importation of virus from endemic areas. It has proven challenging to maintain high MMR coverage, however, and many of the cases detected are in under-vaccinated populations. Also some secondary vaccine failure cases have been detected which will be reported later in the meeting.

In 2014 (to Sept), the USA confirmed 592 cases in 21 states, which was the highest number of cases reported since 1994. A total of 99% were import associated, with 49 separate importations detected and 18 outbreaks confirmed ranging from 3 to 378 cases each outbreak. Of the cases, 93% were unvaccinated or had undocumented vaccination status. Genotypes confirmed to date were: B3 (85 cases), D8 (14 cases), D9 (35 cases) and H1 (4 cases).
CDC also investigated the Federated States of Micronesia measles outbreak, in March 2014 and genotype B3 Harare only found with epi-links to the Philippines outbreak. Of the 289 cases confirmed, 65% of cases occurred in adults > 18 years of age. A mass vaccination campaign is in progress targeting populations from 6 months to 49 years of age.

Four state laboratories in the USA are currently acting as reference centres for the USA and are testing for measles, mumps and rubella.

Genotypes found in 2014 were predominantly B3 (Harare 2009 lineage), D9, D8 and H1. The largest outbreaks were in Ohio (376 cases) caused by D9 and in Missouri (26 cases) caused by B3. One of the reasons for the recent big outbreak in the USA was due to measles getting into communities with high susceptibility (Amish) and thereafter spread very rapidly. There was minimal spillover into the neighbouring community however. US workers supporting the Philippines cyclone in late 2013 became infected with measles and spread it into the USA. There was evidence that the outbreak in the Philippines resulted in the B3 (Harare) virus being widely disseminated throughout the world in 2013 and 2014.

Marilda Siqueira reported the activities of the Brazil RRL in FIOCRUZ.

All 27 states in Brazil have laboratories for MR serology. Any specimen which tests positive or equivocal by the state laboratories are sent to the reference laboratory in FIOCRUZ for confirmation. All Laboratories meet 80% or above for all laboratory specific indicators. All samples are tested for measles and rubella and since 2012, approximately 70% are also tested for IgG.

In 2013, 220 cases were confirmed as measles, with D8 (118 cases), D4 (1 case), and B3 (1 case), genotypes detected. In 2014 (August), 355 cases were confirmed with D8 genotype confirmed in 348 cases and B3 from a small outbreak in Sao Paulo.

As a result of increased measles activity in Brazil, the follow up campaign for < 5 year olds in Brazil will be held in November 2014 instead of as planned for 2016.

Joanne Hiebert reported the activities of the Canadian RRL at the National Microbiology Laboratory (NML), Winnipeg.

Measles and rubella surveillance in Canada (MARS), is a real-time, web-based surveillance model which has been successfully implemented by three provinces and provides real-time reporting of measles/rubella/CRS investigations covering ~25% of the Canadian population.

NML’s role in Canadian Laboratory Surveillance consists of the provision of annual molecular & serological EQA / PT panels to the 26 SNLs. Five SNLs also participate in a measles molecular panel.

Measles and rubella IgG/IgM serology is done at the provincial SNL level and is not routinely performed at NML. However the NML performs specialized serology (rubella IgG avidity, measles SSPE & measles PRNT), measles RT-PCR for provinces not performing it in-house and all Rubella RT-PCR and all measles and rubella genotyping is performed. For measles the H gene is routinely sequenced in addition to the standard N-450 region.
The quality of the Provincial laboratories is good with all Laboratories meeting minimum performance criteria through analysis of proficiency testing panels.

For measles, a total of 83 cases were confirmed in 2013 (D8, B3, H1 and D4) with 9 defined outbreaks, all of which were genotyped (D8 and B3).

In 2014 (up to September 15), 127 cases were confirmed (B3, D8, D9, H1, D4 and D6 [SSPE]) with 18 outbreaks, 17 of which were sequenced. Genotype B3 (Harare) was predominant with epi-links to the Philippines in most. All 35 D8 (Taunton) sequences were identical, with multiple importations from Netherlands. The D9 had epi-links to China. China reported that their D9 viruses were mostly epi-linked to Myanmar.

Two cases of rubella were confirmed in Canada over 2013 and 2014 (to September 15). Genotype 2B was detected with epi-links to Japan in 2013 and 1J was confirmed in a case in 2014 with epi-links to the Philippines.

The Caribbean Subregional activities and challenges were reported by: SueMin Nathaniel-Girdharrie from the Caribbean Public Health Agency (CARPHA), Trinidad.

The Caribbean Public Health Agency (CARPHA) provides laboratory-based surveillance for 24 Member states covering a total population of 17 million. They also support the MR laboratories in Dominica Republic, Nicaragua and Haiti.

In 2013, a total of 346 samples from 11 member states were tested for measles and rubella IgM with no positive cases confirmed. Confirmatory testing of the Dominica Republic, Nicaragua and Haiti laboratories showed greater than 90% concordance for all 67 samples sent.

A large Chikungunya Virus outbreak occurred in the region in 2014 with 1324 positive cases detected. The outbreak appeared not to impact on measles and rubella surveillance as suspected measles case numbers in 2014 were similar to those in 2013, prior to the Chikungunya outbreak.

Joe Icenogle reported on the Developments and Studies carried out by the Rubella Branch of the Global Specialised Laboratory, US CDC, Atlanta.

A preliminary report of US rubella cases detected in 2014 (to August) identified <10 rubella cases and 1CRS case was confirmed but the child was born outside the US (Yemen) and then came to the US. Three cases of rubella were linked to importation, and all were 2B genotype.

The four recently established USA state Reference centres have started testing for rubella but numbers are very low.

Real-time RT PCR was carried out on 9 samples from PAHO laboratories with one positive detected. Eight laboratories submitted samples to CDC for help with classification. Specimens were tested with Diamedix and/or IgG and avidity. Most of the low avidity cases were recently vaccinated.

New developments and studies since the last meeting include: high throughput neutralization method, curation of RubeNS (RubeNS steering committee meeting), potential new real time assay (presented later in meeting).
A new development for semi-automated neutralization testing for antibodies to Rubella virus was discussed. The method is based on a soluble ICA for rubella virus detection in infected cells that allows neutralizing antibodies to be detected in as few as 3 days and eliminates viewer subjectivity. The microformat reduces the amount of serum and reagents required and throughput is enhanced by a factor of about 3 and reduces total technicians hands-on time by a factor of about 6.

Claude Muller presented evidence of the Signatures of the immune response after measles virus infection in the B cell repertoire.

The B cell repertoire after immunisation with measles virus investigated by high-throughput sequencing of the immunoglobulin heavy chain repertoire of transgenic humanized rats. The ability of B cells to respond to an almost infinite universe of antigens relies on the extreme variability of the immunoglobulins (Ig). Ig genes are assembled by stepwise by recombinations of the available Variable (V), Diversity (D) and Joining (J) gene segments, VD and DJ junctional diversity and somatic hypermutations within the CDR regions of the antigen binding site of the antibodies. B cell clones with improved antigen binding affinity survive, resulting in a repertoire of cells with antigen-specific B cell receptors. Advances in high-throughput DNA sequencing technology have made it possible to study the complete repertoire of antigen-specific B cell receptors at unprecedented depth. Since the B cell repertoire is shaped by the antigens encountered, the approach has the potential to retrospectively analyze past immune responses and potentially reconstruct past antigenic challenges. To study the dynamic of the B cell repertoire and to identify specific immunoglobulin signatures against measles virus (MV), rats with a human Ig heavy chain repertoire were immunized with a variety of measles antigens and >200 specific (human) hybridomas were generated. About 1 Mio (human) CDR3 sequences were obtained from the rats’ lymph nodes, the hybridoma library and the hybridoma clones of which about 2800 were unique. 2.5 Mio sequences were obtained from 4 naïve animals 30,000 of which were unique. While the naïve animals shared large numbers of unique sequences between them, less than 1% of these were shared with those of immunized animals. Also, animals that were immunized with other antigens shared very few CDR3s with MV immunized animals. MV-CDR3 patterns were compared with sequences obtained from measles patients and individuals vaccinated with MV containing vaccine. Our studies is that antigen-induce specific immunoglobulin sequences can be recognized on the basis of proliferation and diversity characteristics and that these sequence clusters correspond to complex signatures of MV, that eventually could be exploited to reproduce the immunological history of the host.

Lack of standardization of rubella IgG assays, is there any solution?

A report from Rubella IgG standardization working group made by

Liliane Grangeot-Keros and Christelle Vauloup-Fellous, National Reference Laboratory for Rubella, Virology department, Groupe Hospitalier, Paris-Sud, France.

As response to a rubella IgG external quality assurance (EQA) panel showing wide discrepancy in IU/ml detection in different assays, a Rubella IgG standardization working group was convened. The group has met 4 times since 2012 and has discussed the following issues:

- Has vaccination had an impact on total antibody level?
- Are discrepancies between commercial assays a current issue? Are they due to a lack of specificity (false positive results)? A lack of sensitivity (false negative results)?
- Should cut-off values be identical between assays?
- Is it possible to choose a gold standard (immunoblot, neutralization test)?
- Are low rubella IgG levels protective?
- Is the use of IU/mL necessary?
- Should rubella IgG results be qualitatively or quantitatively reported?

The working group collected data on seroprevalence over time in England, France and Germany. It was found that IgG antibody levels are higher in people born before 1990 compared to those born after 1990 in the UK.

Similar results were found in a German and Norwegian study.

A total of 325 samples that pretested as negative RV-IgG (from France, Italy and Germany) were tested with 8 ELISA assays, 1 immuno-blot, and 1 neutralization assay. It was found that 59% of women considered susceptible have specific anti-E1 antibody and 71% women considered susceptible have neutralizing antibodies.

It was concluded that immuno blots are a reliable standard and seem to correlate to immunity to rubella infection.

On-going action includes: the preparation of a panel of true rubella seronegative samples that will be available for all rubella assay manufacturers to improve the accuracy of their RV-IgG assays.

Vicki Stambos from the WPRO RRL, VIDRL, Australia, reported on the Measles and Rubella IgM Proficiency Testing – Panel 01303.

The 2013 panel consisted of Measles IgM positive (N=6) Rubella IgM positive (N=6), Parvovirus B19 IgM positive (N=1), Dengue IgM positive (N=1), Measles and rubella IgM negative (N=6).

The number of laboratories that participated was 227 laboratories for measles and 223 for rubella over all 6 regions.

Sample 17 and 19 were measles and rubella negative but were found to be positive or equivocal in 18 and 13 laboratories respectively for measles and 11 and 10 laboratories respectively for rubella. For both samples, laboratories in 4 of the 6 regions and excluding AFRO and SEARO submitted discrepant measles results and laboratories in all regions with the exception of AFRO submitted discrepant rubella results. Laboratories had used Siemens but WPR laboratories were most affected. No cause could be identified for the discordant results so scores were adjusted by excluding the discordant results for samples 17 and 19.

Overall results saw 99% of laboratories pass the measles PT (91% with 100% score) and 100% of laboratories passed the rubella PT (93% with 100% score).

A small percentage of laboratories did not comply with providing validation criteria and 78% reported results to VIDRL within the required 14 days.

It is expected that laboratories meet 3 main criteria for passing the PT, test validity data provided, score ≥90% and results reported within 14 days.
An update on the Measles/rubella serosurvey literature review and development of Serosurvey Guidelines was presented by Mick Mulders

Mick Mulders reported the progress on the development of Serosurvey Guidelines being developed by WHO Consultant Ray Sanders in a BMGF funded project. The guidelines are being developed based on analysis of Medline literature search for the years 1999-2014. A total of 61 measles and 55 rubella papers were reviewed.

A small working group has been established to develop the guidelines that will be developed under the broad sections:

1. Introduction
2. Design and planning (including ethics)
3. Survey implementation
4. Laboratory methods
5. Analysis, conclusions, reporting, feedback

The guidelines will be supplemented by a reference section and a series of annexes.

It is proposed that the draft guidelines will be completed by November 2014, testing will occur in early 2015 in a pre-campaign setting in Uganda and Ethiopia and in a post-campaign setting in Zimbabwe and Cameroon. A “Final” draft should be available by the end of 2015.

A presentation on Results of serosurveys carried out in 2013/2014 by WHO was made by Robert Perry, WHO/HQ.

Dr Perry described what population immunity was and why measuring it is important in determining population susceptibility or immunity profiles in order to target immunisation activities.

Nepal held a measles and rubella campaign 2012/13 in three phases with reported coverage high but the denominator not always accurate. A serosurvey was completed to evaluate the outcomes of the campaign and the resulting levels of immunity to measles and rubella.

The number of children surveyed totalled 7753 with OF collected from 2510 and serum from 926. Laboratory analyses were done in the Nepal PHL in Kathmandu and 10% of results were confirmed at Thailand NIH. Cut-offs for immunity used were 120 mIU/ml for measles and 10 IU/ml for rubella.

The Nepal MR campaign reached 93% coverage by survey and serology testing showed 91.7% were above the immunity cut-off for measles and 94.3% for rubella.

OF collection was well accepted and serum acceptance was lower, however, OF sensitivity was 51% and specificity of 33% compared with serum. Kappa agreement score was 8%, indicating poor OF correlation with serum. Additional analysis of the OF samples and results are planned by PHE. However the main conclusion is that OF is not suited for serosurveys. Additionally the Rubella IgG kit for testing OF samples was taken off the market right before the start of the study.

A number of lessons were learned from the study, including that training of
laboratory staff on the management of large numbers of specimens and data, an on-site assessment of laboratory is important and on-site training is critical. It was also recommended that a full-time local coordinator should be appointed during the entire study and analysis phase.

A presentation on *Serosurveys 2013/2014 – preliminary results and status*, was made by Jim Goodson, US CDC.

CDC has been involved in a number of serosurveys in Cambodia, Namibia, DRC, Angola, Myanmar, Nepal, and Jordan over the past 8 years. The experiences gained from these assessments were discussed.

Nationally representative serosurvey estimates by age group are useful for:

- Rubella vaccine introduction planning
- Measles SIA planning for elimination
- Disease modelling to predict outbreak epidemiology and immunity gaps for guiding elimination efforts

However, to guide disease programmes, results need to be available and communicated in a timely manner. Results should also be triangulated with surveillance and vaccination coverage data to manage population immunity.

He suggested that alternative methods to sera and OF may prove more useful and should be evaluated, including using DBS tested with Luminex technology.

David Brown, PHE, UK, presented an update on the use of Point of care tests for Measles IgM and IgG.

A point-of-care test (POCT) for measles diagnosis and the detection of measles-specific IgM antibodies and viral nucleic acid has been developed by PHE and is being scaled up with a commercial company. BMGF has funded a project to provide a rapid, inexpensive and sustainably produced measles and tetanus IgG tests that provide unambiguous results at the point of collection using OF or finger prick blood.

The Oracol device which has been used for MR surveillance in UK for 20 years is not suitable for population base sampling and the OF collection device has been re-engineered to allow collection and extraction of fluid within the same device and will be ready for evaluation within the next 2 months.

Evaluation of the POCT was carried out by collecting OF and serum paired samples from two groups and tested for Tetanus and measles for 12-59 months age and 3 month age from a study in Entebbe Uganda. Evidence was shown that 95/113 (84%) children 4-6 months of age were seronegative for measles IgG and 25/203 (12.3%) children 12-75 months of age did not have protective levels of antibody.

The POCT on OF samples for tetanus immunity is work in progress but is showing promising results in comparison with EIA on OF (sensitivity 93.1% specificity 98.5%). A larger batch of POCT is currently being built to enable further optimisation.
Fiona van der Klis, RIVM, The Netherlands, presented an **update on Multiplex high throughput serology**.

The advantages of multiplex testing using the Luminex platform were described which included: good sensitivity and specificity compared to EIA, low volume use of antigen and antibody, reduced labour costs and cost-effective vs. ELISA using 3-plex antigen (MMR). IgG and IgM and avidity can be measured also.

RIVM has conducted two large population based studies, monitoring national immunisation programme and identifying risk groups and monitoring waning immunity.

They have also investigated using dried blood spots and oral fluid samples in place of serum/plasma. Good correlation between plasma and DBS was found and little difference was detected between venous and finger prick blood. Oral fluid needs optimisation however, as at low levels of antibody OF appears to generate non-specific binding.

The Results of **high throughput serology: Measles antibody detection by EIA (Luminex): correlation with immune protection** was presented by Rob van Binnendijk, RIVM, The Netherlands.

Plaque reduction neutralisation test (PRNT) is currently the best standard for measuring humoral protection in measles, however, it is labour intensive and intra-test and inter-test variability exists. Few laboratories in the world routinely use PRNT but use commercial EIA (IgG) testing for measles immunity testing.

RIVM has evaluated the correlation of EIA (Luminex, Siemens and Vidas IgG assays) with PRNT. Siemens and Vidas had lower sensitivity than PRNT but Luminex showed better correlation although some lack of sensitivity was also detected.

**Molecular EQA: Results from FTA Practice Panels and Initial Molecular PT Panel** were presented by Paul Rota, US CDC, USA.

The results and conclusions of the FTA molecular practice panels distributed to laboratories after molecular training workshops or from regional offices for the past several years were reviewed for the 206 laboratories that participated. It was reported that the genotyping RT-PCR assays performed well and almost all laboratories reported the correct results with minimal cross contamination in standard, endpoint RT-PCR assays.

The next steps in implementing a regular and universal molecular PT programme for measles and rubella was described. The protocol has been finalised, laboratories identified (up to 4 per region), SOPs for panel production developed. Panels were shipped to 22 laboratories in March 2014 and analysis of results fed back to laboratories by June 2014. The results of the first PT panel were that all laboratories passed, one on retest. One laboratory did not respond.

Some of the lessons learned from first panel were: shipping was still challenging even though panels were non-infectious and required no dry ice, quality of the reports varied, there were few errors in sequencing reactions and most sequences were identical for the same sample between laboratories, and some laboratory process errors were detected. It was stressed that laboratories need to use the supplied forms and return result as MS Word files to make it easier for the analysis.
There are a number of issues to finalise before the next panel is distributed in 2015 and participants are requested to respond to the questions posed by the presenter.

The outcomes of the first measles PCR ring trial in Germany, Instand e.V. were presented by Annette Mankertz and Sabine Santibanez, RKI, Germany.

Dr Mankertz reported on INSTAND, an external Quality Assessment Scheme in Germany. There are two schemes relevant for the LabNet, one for molecular techniques and one for serology. As examples, one recent panel was described that was developed to evaluate detection of the measles virus genome in 29 laboratories in the country. The panel consisted of four samples, 3 with different genotypes and one negative. Twenty-five of 27 (92.6%) laboratories gained 100% score. Panels for evaluation of measles IgG/IgM detection have been in place in Germany since 1994 and are distributed twice a year. A recent example panel consisted of two samples, one IgG positive and IgM negative and the other IgG positive and IgM positive. Laboratories were expected to evaluate IgG, IgM and avidity (if used). Of the 240 laboratories that evaluated the panel, 98.7% correctly identified the IgG and 99.1% the IgM. All six laboratories that tested for avidity were 100% accurate.

Dr Mankertz reported that INSTAND would be willing to contribute to the molecular EQA programme for the WHO LabNet, either regionally or globally. It was suggested that INSTAND could be used in the European region especially with so many laboratories, however, WHO may need to contribute to technician time (about ¼ FTE) in developing the panels.

Richard Meyers, PHE, UK, presented an update of the RubeNS rubella sequence database.

Currently RubeNS contains 1078 sequences with the majority assembled from GenBank submissions. It was reported that there are very few submitting users. Only about 30 sequences per year are submitted and are probably not all the rubella sequences detected in the LabNet.

The formulation of the WHO name is critical to identify the sequence and the website will reject any data discrepancies submitted. For inter-database comparison (RubeNS and GenBank), the genotype identifier used in MeaNS will not be stored within the WHO name in RubeNS.

A phylogeny tool is now available on the RubeNS website utilising the 32 reference sequences over the 739 bp fragment of the E1 gene. The collaboration with CDC and PHE continues to actively develop RubeNS, in particular, web code changes and database curation.

Predominant genotypes found recently are 2B and 1E with most 2Bs reported from WPR, reflecting the large volume of sequences submitted from this region. Genotype 1E is predominantly reported from WPR and some EUR countries.

Richard Meyers, PHE, UK, presented an update of the MeaNS measles sequence database.

Currently 19033 N450 sequences have been submitted to MeaNS and 817 full-length H gene sequences. More than 4000 sequences have been submitted in past 12 months with real-time submission by China now occurring. Fewer sequences were reported from EUR in 2014 than in the past. B3 has been the predominant genotype found by country and region, most identical or closely
related to MV/Harare.ZWE/38.09 (B3 Harare). A total of 1031 sequence of B3 Harare have been submitted to MeaNS over the past 5 years.

Some of the changes to the functionality of MeaNS in the past 12 months include: a “flash” screen used to remind submitters about various issues when using the website, “named” strains are sequences seen frequently and are useful identifiers (e.g. B3 Harare, D8 Taunton). Users are encouraged to contact the curator if they feel any strain is appropriate for “naming”. Additional tables have also been added and include monthly summaries, NL summary, country listings and it is possible to select a summary of submissions for a range of years. Users are encouraged to feedback any comments to the curator.

Janice Lo, Hong Kong RRL, reported on the genotypic analysis of the Philippines measles outbreak, 2014.

The Philippines experienced a large measles outbreak in 2013-2014 affecting most of the country. Reports were received of genotype D9 measles in Ohio in April 2014 with epi-links to the outbreak in unvaccinated persons helping build houses in the Philippines following the typhoon in November 2013. The same D9 sequence was identified from 4 cases and had the same sequence as a case in Hong Kong with unknown epidemiological links and strains from Fukuoka in Japan. However only one D9 sequence was identified from cases in the Philippines (region 7), in January 2014, which was one nucleotide different from the Ohio and Fukuoka cases. The sequence was different to the previously endemic D9 strain (2012) in the Philippines. Conversely, genotype B3 (Harare) was detected from the majority of cases in the Philippines, 40 sequences from 13 of 17 regions. Sixteen B3 viruses were detected from region 7 affected by the typhoon as well as one D9.

Vicki Stambos presented Molecular Surveillance Of Measles Viruses In Australia in collaboration with Thomas Tran, VIDRL, Australia.

The Western Pacific Regional Verification Commission verified that Australia has interrupted endemic measles virus transmission for a period of at least 36 months since 2009, a decision based in part on molecular surveillance data provided by VIDRL from 2008-2012. The pattern of genotypes found in Australia over this period was typical of multiple importations, limited circulation of strains and no evidence of the circulation of endemic strains was found. A total of 85% of confirmed cases were imported or linked to importation and the confirmed cases of unknown source had sequences indicative of importation.

In 2013, Australia reported 75 measles cases detected through molecular surveillance and 155 cases in 2014. Four wild type genotypes were confirmed in 2013, (G3, D9, D8 and B3) with D9 predominating. In 2014, 5 genotypes were confirmed, (H1, G3, D9, D8 and B3) with B3 predominating. At least 10 countries were identified as possible sources of importation and 25 separate importations of genotype B3 were confirmed from the Philippines.

Said Al Baqlani, Oman RRL, reported molecular surveillance results from EMR member states supported by Oman, 2013-14.
Oman detected 7 viruses in 2014 and all were related to importation. Genotypes B3 were confirmed in Oman, Bahrain, Iran, Saudi Arabia, and United Arab Emirates. D8 was detected from Syria, Lebanon, Jordan, Bahrain, Oman and Iraq. Rubella genotypes detected were 2B confirmed in Oman, Bahrain and Iran.

Some Member States in the region were reported to be reluctant to submit sequence information to MeaNS and RubeNS.

An update on the progress with the Verification of measles elimination in Japan was presented by Katsuhiro Komase, NIID, Japan.

Japan is considered under WPRO’s verification classification as “May be ready for verification but additional information needed”.

The current status of measles in Japan was reviewed. Evidence shows that measles incidence over the period 2008-2014 has shown considerable decline. In 2013 and 2014 many importation cases were detected, mainly from the Philippines and in 2014 (to August), 436 cases have been confirmed with 79% also having genotyping evidence, a big improvement from the approximately 20% of confirmed cases with sequence information found in previous years.

Genotype B3 predominated in Japan in 2013-2014, making up 50% of cases in 2013 and 70% in 2014. Most are variants (N=14) of B3 Harare. D8 was the second most common genotype found, with 7 clusters detected. There has been an increase in the proportion of laboratory confirmed measles cases with genotype results and fewer laboratory confirmed cases without genotyping or clinical cases classified.

Genotype D5, which was considered the endemic virus in Japan during 2006-2009, has not been detected since May 2010. There is no evidence of any virus strain to have circulated for more than 1 year since then. Japan is considered to be close to achieving measles elimination as they are reporting high vaccination coverage and sero-prevalence rates of greater than 90% (and most over 95%) across all age groups except those under 1 year of age.

A presentation entitled Virological Surveillance for Measles in Mainland China associated with predominant and endemic H1 genotype MeV was made by Zhang Yan, RRL China CDC, China.

Measles cases in China were at their lowest level in 2012 after the nation-wide campaign in 2010 but there has been a resurgence in 2013 and 2014.

A total of 5944 measles virus sequences were obtained during 2013 to 31 Aug 2014 with genotypes: H1 N=5826, D8 N= 48, and D9 N=58, B3 N=11, and G3 N=1. H1 continues to be the predominant genotype and is widespread throughout China, however, imported viruses (D8, D9, B3, G3) were detected in 7 provinces in 2013 and 5 provinces in 2014. Representative viruses have been submitted to MeaNS prior to January 2014 but subsequently, every sequence has been submitted to MeaNS.

Several imported outbreaks were reviewed. A D8 outbreak was reported in adults attending two big Beijing clothing wholesale markets from March to July 2013. The virus had 99.8%-100% nucleotide homology with viruses circulating in Russia, France, Canada, Thailand, Denmark, and Germany. The
virus subsequently spread to Inner Mongolia and Hubei provinces. More than 97% of cases were >15 years of age.

D9 virus continues to be found in China with most having epi-linkages to Myanmar. Some local transmission has been detected, including 4 secondary cases detected in Shandong in 2013.

B3 virus was found in Shanghai in September 2013 for the first time in China. Since then, identical B3 strains were found in several provinces. Insufficient epidemiological data is available to support epi-linking of cases.

In summary, endemic H1 viruses were associated with the resurgence of measles in Mainland China in 2013 and 2014. Several non-H1 genotype viruses were imported and spread to several provinces in China.

Zhang Yan, China CDC, Beijing, China, reported an investigation of the Genetic Diversity and Evolution of genotype H1 measles viruses in China from 1993-2012.

Using Phylogenetic analysis, Bayesian skyline plots analysis and BEAST analysis, a study was carried out to analyse the transmission patterns of endemic genotype H1 strains, to measure the impact of SIAs in China, to analyse the genetic divergence and evolution of H1 measles viruses over time, to estimate the most recent common ancestor (TMRCA) for genotype H1 and calculate the nucleotide substitution rates for H1 MeV genes.

The analysis was carried out by China CDC in collaboration with US CDC staff and included a dataset of viruses including: 175 N gene, 86 H gene and 69 F gene sequences. It was concluded that transmission of most H1 measles viruses were interrupted by immunization activities and the genetic diversity of H1 viruses in China decreased following 2005, which coincided with a significant reduction in the transmission chains of measles virus. Also the expanded vaccination programme of SIAs in 27 provinces during this period and a nationwide SIA in 2010 may have contributed to the reduction in diversity.

Patcha Incomserb, RRL, NIH, Thailand, presented a Measles Genotype Update for the SEAR.

During 2013 to 2014, the following genotypes were detected in the SEAR: B3, – D8 and D9 in Thailand, D4 and D8 in India, and D8 and D9 in Indonesia. Indonesia has not provided sequence information, however.

The D4 genotype from India was made up of two lineages. Most of the 2013 and 2014 strains were classified as a unique group that differed from Nepal and D4 Enfield, except for one strain from Thane, which was belonged to a Nepal cluster. India viruses were similar to strains reported from Oman, USA and Canada.

The D8 genotype circulated in India since 2002 to 2014 has been classified into three lineages. Most of the 2013 and 2014 strains were classified to two groups, group I and group II. The group I seem to be circulating in India and differed from Nepal, Thailand and Sri Lanka. While the group II are highly similar to the strains found in 2002 to 2012.

The D8 circulating in Thailand in 2013 was highly similar to the strains found in 2011 and 2012.
The D9 Thailand outbreak in 2013 originated in a Myanmar Refugee camp and subsequently was found in three provinces bordering Myanmar.

The B3 genotype was found for the first time in Thailand in 2014 and strains were very similar to the named strain “Harare 09”. B3 was also confirmed in India in 2012.

It was reported that SEAR still have problems of getting genotype information from all countries in the region. However, there was progress with getting information from India but sequence data from Indonesia is still awaited.

Kevin Brown on behalf of Judith Hübschen, Sabine Santibanez and Sergei Shulga presented an update on measles genotypes in the European region.

It was reported that the following information for the region was summarised from viruses submitted to MeaNS and may not be fully representative of viruses circulating in the region.

Three measles genotypes predominated in EUR in 2013 to 2014 (September), D4, D8 and B3. The D4 genotype that predominated in the region between 2010 and 2012 has not been reported so far in 2014. D8 predominated in MeaNS submissions in 2013 and has been reported with similar frequency to B3 in 2014.

B3 “Liverpool” which caused a large outbreak in the UK in 2012 has been replaced by B3 “Harare” and “Tonbridge” strains. The B3 “Harare” strain has been reported from 13 countries in the region in 2014.

Three D8 strains predominated in the region over 2013-2014: “Villiparum” (9 countries), “Frankfurt” (22 countries) and “Taunton” (14 countries) which also were found in most of the cases in an outbreak in the UK.

More than 100 cases in the region were associated with importation based on information submitted to MeaNS.

Joe Icenogle, US CDC, USA presented comments about Rubella Genotyping.

Rubella virological surveillance and genotyping remains a challenge in the LabNet. However there are examples of encouragement as in the last few months at least 3 papers have been published containing analysis of rubella viruses from three countries, China, Uganda and Viet Nam.

The Ugandan paper was from collaboration between Uganda Virus Research Institute and US CDC and resulted in the analysis of 20 rubella viruses collected between 2003 and 2012 in Uganda. The predominant genotype identified was genotype 1G, with only one 1E virus identified. Genotype specific trees showed that the Uganda viruses belonged to specific clusters for both genotypes 1G and 1E and grouped with similar sequences from neighboring countries but the 1G viruses were separate from other geographic regions. More virological surveillance is needed to determine if genotype 1E, which was identified in a border district, is also endemic.
Zhen Zhu presented the evolutionary analysis of rubella viruses in the mainland of China in collaboration with Wenbo Xu, China CDC.

Nation-wide rubella surveillance in China has yet to be established, and most of the provinces conducted laboratory detection only for the differential diagnosis of measles. Therefore, reported rubella cases are mainly comprised of clinically diagnosed cases.

From January to June 2014, a total of 9401 rubella cases were reported, and numbers reached the lowest level detected in the past 10 years. However, the epidemic rubella cycle in China is about every 7 to 8 years and the epidemiology of rubella may have changed since the introduction of rubella containing vaccine in 2008.

A total of 1238 rubella isolates have been obtained since virological surveillance was initiated in 1999. Four genotypes, including, 1E(958), 1F (15), 2A(3), and 2B(262) have been detected since 1999. During 2013 to 2014 (September), both genotypes 1E and 2B were detected. 1E virus found during 2011-2012 continues to circulate in 2013 and 2014. A new lineage of 2B was introduced into China in 2011 and continues to circulate through 2013 and 2014, moving to Western provinces. From 2009 to 2010 a rapid increase of genetic diversity occurred, which was most likely due to improved surveillance. However, the genetic diversity has remained stable since 2010, which likely represents the baseline of genetic diversity of rubella virus in Mainland China.

Yoshio Mori, NIID, Japan, presented an update on the molecular surveillance of Rubella in Japan.

Japan experienced a large rubella outbreak in 2013. More than 14000 cases were reported in 2013, and 268 in 2014, with 40 cases of CRS detected. The outbreak occurred mostly in males (77% of cases) mainly because 35-51 year-old males had no opportunity for rubella vaccination as rubella-containing vaccine was only offered to girls until 30 years ago. Molecular surveillance has improved since 2013, with approximately 50% of cases genotyped.

During 2013-2014 more than 70% of cases were laboratory confirmed and the remainder clinically diagnosed. Approximately half of the cases had genotype information identified. In 2011, 2B and 1E were found in approximately equal proportion. In 2013, 2B predominated (approximately 90%) and in 2014 all 9 viruses detected were 2B.

The sequence of the 2B genotype over 2013 to 2014 was different to previously circulating strains and was related to strains found in South East Asia-East Asia.

An update on the rubella genotype in the SEAR was provided by Patcha Incomserb, NIH, Thailand.

Only six of ten countries in the region have reported rubella genotype information; Bangladesh, India, Indonesia, Nepal, Sri Lanka and Thailand between 2005 and 2013. Only genotypes 1E and 2B have been identified in the region. Genotype 1E was found in Indonesia, Sri Lanka and Thailand and 2B in Bangladesh, India, Indonesia, Nepal, Sri Lanka and Thailand.

Sergei Shulga gave a report on rubella genotype distribution in the Russian Federation and NIS region.
It was reported that data on rubella virus genotypes in the NIS sub-region are very limited. There has been no data on transmission of the previously endemic 1H genotype since 2010 and a series of rubella outbreaks in the Russian Federation over 2011 to 2014 of 2B and 1E “Asian origin” genotype strains were associated with importation and resulted in limited local spread. Genotypes 2B and 1E had relatively high diversity which confirms their independent importation from different sources.

An update on the use of FTA cards for measles surveillance was provided by Paul Rota, US CDC. Samples from DRC are being collected for evaluating FTA cards for direct sampling for molecular surveillance. Samples have been collected from measles and rubella cases and spotted onto FTA cards and shipped to CDC for analysis. Results are still pending and will be reported in the Global meeting next year.

A presentation on Secondary Vaccine Failure for Measles was made by Paul Rota, US CDC. A summary of an outbreak of measles in New York City in 2011 among persons with prior evidence of immunity was presented. There has been evidence of secondary infection in the past but this is the first time spread of infection has been documented.

Suspected cases and contacts exposed during a measles outbreak in New York City in 2011 were investigated. Medical histories and immunization records were obtained. Cases were confirmed by detection of measles-specific IgM and/or RNA. Tests for measles IgG, IgG avidity, measurement of measles neutralizing antibody titer, and genotyping were performed to characterize the cases.

The index case had two doses of measles-containing vaccine. Of 88 contacts, four secondary cases were confirmed that had either two doses of measles-containing vaccine or a past positive measles IgG antibody. All cases had laboratory confirmation of measles infection, clinical symptoms consistent with measles, and high avidity IgG antibody characteristic of a secondary immune response. Neutralizing antibody titers of secondary cases reached >80,000 mIU/mL 3-4 days post-rash onset while that of the index was <500 mIU/mL 9 days post-rash onset. No additional cases occurred among 231 contacts of secondary cases.

This is the first report of measles transmission from a twice-vaccinated individual. The clinical presentation and laboratory data of the index were typical of measles in a naïve individual. Secondary cases had robust anamnestic antibody responses. No tertiary cases occurred despite numerous contacts. It was noted that IgG levels rise very quickly and if paired sera are used for diagnosis then there is a need to collect the first sample very early after disease onset.

The preliminary results of next generation sequencing for measles virus were presented by Judith Hubschen, CRP, Luxembourg.

Data from analysis of 58 D4 “Manchester” measles samples from Belgium (N=25) and Germany (N=33) previously reported where all viruses had identical N450 sequences and were subsequently sequenced over the complete P and H genes.
The P gene sequencing revealed 11 sequence variants and a maximum of 6 nucleotide differences. The H gene sequencing revealed 9 sequence variants with a maximum of 5 nucleotide differences. A combined N450PH tree identified 16 sequence variants and a maximum of 9 nucleotide differences.

At least one strain per cluster was sequenced using new generation sequencing (NGS). NGS data included the L (6552 nts) gene and F gene (1653 nts) and analysis of 13164 nts identified the same 8 clusters but with 26 nts maximum distance.

In conclusion, the clusters in the N450PH sequence analysis were confirmed in individual genes and in the overall data. Nearly full-length data provided by NGS provided better bootstrap support but did not seem to provide new insights into mutations or clustering.

Richard Myers presented the whole genome sequencing (WGS) of MeV from clinical samples in collaboration with Ana Penedos and Kevin Brown, PHE, UK.

Evidence was provided of 3 attempts of random primer amplification using Roche 454 which showed limited success on cell culture supernatants (<40% of genome) and no success with oral fluid samples. RT-PCR results of 9 overlapping primer pairs covering the whole genome with primers designed to cover D4 and D8 genotypes provided good amplification in tissue culture fluids and gave low sensitivity with oral fluids. RT-PCR results of 20 overlapping primer pairs covering the whole genome designed for B3, D4 and D8 genotypes gave good amplification of tissues culture supernatants and was successful on oral fluids samples with > 7.5 genome copies.

New generation sequencing using Illumina MiSeq was used to look at 12 genomes already completed by Sanger sequencing, 3x B3, 4x D4 and 5x D8 sequences. Results of 5 full genomes and 29 with non-coding gaps and 27 with non-coding and other gaps show perfect match between sequences by Sanger ND NGS.

For isolates with identical N450, were found to have >40 differences, in the approximately 14.5 kb sequenced thereby providing added resolution. It was calculated that measles virus has a substitution rate of approximately 1.7x10⁻³ substitutions/site/year.

In summary, for 42 samples tested, WGS provides additional resolution compared to looking at N450 alone. However the added data can be potentially more difficult to interpret without analysis of a more comprehensive library of other sequences to determine possible origins.

Data is from oral fluid samples and may be better to analyse from isolates.

As with other methods, there were challenges in sequencing the non-coding region.

A presentation on measles whole genome sequencing and vaccine-specific RT-PCR was made by Alberto Severini, from Public Health Agency of Canada.

Measles genotyping from 2011 to 2014 identified issues as a result of recurring introduction of similar genotypes from global outbreak areas. This lack of resolution with the N450 region has reduced the power of distinguishing importations from local transmission.

Whole genome reverse transcription was completed using cDNA amplification by 6 overlapping primer sets using Sanger or next generation sequencing.
More than 80 viruses were analysed which were identical for the N450 and complete H gene. Three clusters were identified from full genome analysis, which identified outbreaks following separate importation events. A similar pattern was found with the M/F non-coding region (NCR). It was considered that M/F NCR could be used as substitute for full genome with similar resolution, and a faster and perhaps more economical sequencing than WGS.

Some provinces in Canada regularly request next-day genotyping of suspected vaccine-related cases as rapid detection of a vaccine genotype in suspected cases of measles is often crucial for an appropriate public health response. Such a rapid turnaround time for sequencing is sometimes difficult to deliver however. In response, a rapid qPCR test to detect vaccine genotypes was developed. Results of the analysis of 53 A strains using vaccine specific primer probes provided 100% sensitivity and 100% specificity.

As Canada found that the CDC probe was not detecting B3 Harare H gene some provinces were reporting “equivocal” results (Positive N gene, negative H gene). In response, a new probe for real time PCR was developed which allowed detection of B3 H gene.

Joe Icenogle reported progress with the investigation of using a new window to detect rubella virus in collaboration with Min-Hsin Chen and Emily Abernathy, USCDC.

The region in the current window (in E1) has high nucleotide heterogeneity compared to the 5’ end of the genome and not all regions with conserved oligo sequences are good for detection, e.g. the conserved region in E1. The current detection window has failed to detect a few cases, which either have very low RNA or are some specific clade 2 viruses. Multiplexing might eliminate separate diagnostic assays for the detection of rubella virus and the cell reference gene.

Any new assay would optimally have greater sensitivity for detecting current predominant circulating genotypes (e.g. genotype 2B) and need to have as little modification as possible –protocol, instrument, reagents, and be similar to the measles RT-PCR assay.

A good window in NSP for detecting rubella virus was found and the NSP assay was analytically more sensitive on all genotypes. The NSP assay was specific for RV and showed some improved clinical sensitivity than the current E1 assay. It is being considered to multiplex the detection of RV and a reference gene.
Serology

1. All the WHO Regions have adopted goals to eliminate measles. One of the criteria to verify elimination is documenting high population immunity, which can be assessed by seroprevalence studies. The meeting recommends developing a working group to evaluate seroprevalence studies and laboratory methods for measuring antibodies to measles and rubella. This working group will advise the working group which is developing guidelines on assessing population immunity against measles and rubella, funded by the BMGF.

2. As measuring serum antibody levels in population-wide studies has been recognized as a useful tool to assess the progress of measles and rubella elimination there is growing need for rapid and high throughput technology for determining IgG titers. Therefore, the participants recommend that GMRLN explore the use of alternative methods other than conventional/commercial EIA, such as multiplexed immunoassay (MIA) based on Luminex technology, high throughput neutralization assays, and point of care assays, for the feasibility to assess seroprevalence.

3. Serum specimens for evaluation of serologic assay kits and for quality control purposes should have detailed epidemiological and laboratory backing (e.g. PCR results, IgG, IgG avidity). Preferably, serum specimens should be included from e.g. reinfection cases and cases of other rash causing illness. See point 4 below.

4. The meeting participants suggested a reevaluation of the performance of current IgM and IgG assays, especially because of concerns with interpretation of results in cases of secondary vaccine failure. LabNet should support laboratory studies on the relationship between the performance of EIAs and the vaccination status of the case. The key findings, particularly concerning assay performance characteristics (sensitivity, specificity, applied cut-offs, reproducibility, etc.) should be shared within GMRLN and published.

5. [deleted]

Molecular analysis

5. One of the three essential criteria for verifying elimination is genotyping evidence to support the interruption of endemic transmission. All network laboratories should encourage and assist with the collection and analysis of appropriate clinical specimens for genotype analysis. Sequence data should be submitted by the national laboratories to MeaNS and RubeNS in a timely and complete fashion. The laboratories are requested to submit all sequences, rather than a selection of sequences for WHO to obtain a better overview of the measles and rubella situation in its Member States. Sequences can be uploaded batch-wise. WHO can assist in obtaining the proper permissions from national authorities for national laboratories to upload to MeaNS/RubeNS.

6. Based on the epidemiological and molecular characteristics of a certain virus variant, the variant may be nominated as a “named” strain, and to be used in describing outbreaks and use them in presentations and publications. Therefore, laboratories are requested to use “named” strains for describing virus lineages. Laboratories are also requested to nominate named strains that represent significant outbreaks or extensive transmission chains.
7. To facilitate uploading sequences to MeaNS and to accommodate challenges in obtaining sufficient epidemiological information RRLs can submit sequences to MeaNS on behalf of a NL using specifically developed submission tools to facilitate sharing genotype information with the global program in a timely manner.

8. With the increased usage and necessity to produce regular Regional reports, the participants recommended providing a tool to countries for extracting epidemiological data from MeaNS and RubeNS which will subsequently aid the verification of elimination (e.g., frequency of detection of named lineage versus epi week).

9. The meeting noted the need for virus isolates with the development and implementation of next generation sequencing technology and its application to genetic characterization measles and rubella viruses. Therefore, network laboratories are kindly requested to submit viral isolates to the WHO strain banks. The GSLs are to make an inventory of current isolates that have been submitted and determine which ones would be useful for sharing with GMRLN.

10. The current genotyping approach for measles is based on sequence analysis of the 450-nucleotides coding for the 3'-terminus of the N-gene. This strategy is currently sufficient for routine genotyping in GMRLN. An ongoing research activity of selected reference laboratories is to determine the benefit of using a longer sequence window for molecular epidemiologic analysis to provide a more detailed understanding of viral transmission pathways. Furthermore, the H-gene sequence data will provide important information on putative antigenic variation in measles H.

11. The meeting recognizes the importance of next generation and full genome sequencing. In order to streamline and coordinate activities, a small working group should be developed.

12. With measles incidence gradually decreasing, and more laboratories conducting molecular diagnosis, rapid differentiation between vaccine reactions and wild-type measles virus infections would be a useful tool for the laboratory especially in elimination settings. The meeting recommends making the vaccine-specific PCR protocol as developed by PHA Canada available to GMRLN when it is fully validated.

Surveillance

13. It is of critical importance that specimens for serological but particularly for molecular analysis is accompanied by detailed epidemiological information to ensure proper interpretation of the molecular data, including defining the source of importation. Therefore, laboratories are requested to support collection and submission of epidemiological data.

14. The meeting recognizes the implications of new technology on the quality of surveillance data, guidelines, SOPs, assay controls and urges GMRLN to address these. The current version 2 of the WHO Laboratory Manual for Diagnosis of Measles and Rubella needs revision to address new technological developments, expansion of quality control measures, and the challenges of performing laboratory confirmation in an elimination setting.

15. In the EUR and EMR, the FTA cards to transport samples for virologic detection has successfully been used to enhance measles and mumps molecular surveillance in several countries that rely on external sequencing capacity. A protocol for application of clinical samples to FTA Classic Cards and extraction of viral RNA from the cards will be prepared by RRL Berlin (RKI) for sharing within GMRLN by the end of 2014.
Quality assurance

17. The serologic proficiency test program run by the Victorian Infectious Diseases Reference Laboratory in Melbourne has become a core activity of the GMRLN EQA program with over 200 laboratories now participating. To add an extra level of stringency, scoring of the proficiency test results should now include not only results, but also timeliness and completeness of assay data and assay validation data.

18. The meeting agrees that the initial molecular proficiency test panel introduced to 22 network laboratories in 2014 was a success. However, this quality control program needs to be expanded and improved. To help guide the molecular PT program for 2015, a survey will be distributed to LCs, GSLs, RRLs to address the following issues:
   a. Frequency of testing (annually, biannually)
   b. Number of laboratories to be assessed in each region
   c. Composition of molecular PT panels
   d. Time frame for distribution of samples and reporting results
   e. Supporting data to be included in the report
   f. Reporting format
   g. Regional production and distribution of panels

19. Additionally, further development of the mEQA program is needed including addressing stability of viral RNA on FTA cards, and involving other organizations like the German INSTAND e.V. to produce the molecular PT panels.