Report on the
10th WHO Global Measles Rubella Laboratory Network Meeting
WHO Headquarters, Geneva, Switzerland
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Rapporteurs: J Leydon, Dr J Goodson

Introduction

The WHO Global Measles and Rubella Laboratory Network performs a key role in measles and rubella surveillance by confirming suspected cases using standardized and validated testing and reporting procedures. The WHO Measles and Rubella Laboratory Network (LabNet) comprises 690 laboratories globally, almost all of which are following standardized testing and reporting procedures and undergo regular quality assurance and proficiency testing assessments. Representatives from key specialized and reference laboratories within the LabNet and the WHO laboratory staff responsible for the coordination of the LabNet meet annually in Geneva. In 2012, the meeting discussed in 8 sessions the progress within the network, enhancing surveillance for measles and rubella, quality assurance and standardization, the challenges of meeting elimination criteria for measles and rubella, the utilisation of additional tools for IgM testing to support surveillance, tracing measles virus transmission pathways and proposals for improving surveillance performance and an update on mumps nomenclature and diagnosis.

Meeting objectives

The objectives of the meeting were:

- To review and discuss the current status and management of the Global Measles and Rubella Laboratory Network in order to develop and strengthen the technical capacity and structure of the network.

- To provide WHO staff and Measles and Rubella LabNet representatives with technical updates on the Laboratory issues related to measles, rubella and mumps control, and quality assurance of the LabNet.

- To determine how best to meet future challenges for the measles and rubella network

Expected outcomes:

- Technical recommendations and a plan of action for further development and strengthening of the Measles and Rubella (and Mumps) LabNet for 2012-2013 will be developed.

Session 1: Opening

In his welcome speech Dr Peter Strebel expressed apologies for the Director Dr Jean-Marie Okwo Bele who was unable to attend the opening session of the meeting. He highlighted the fact that for the
The first LabNet meeting held in 2001 was one page, and in 2012 we have a five-page agenda and a busy three-day meeting. At the World Health Assembly held in May 2012, monitoring and accountability of measles vaccination was highlighted as an important issue. CDC and Bill Gates are increasingly seeing the relevance of a monitoring program.

**Session 2: Global & Regional Briefings**

An update on the global Measles and Rubella elimination strategy was provided by **Dr Peter Strebel**, who leads the Global Measles Control Programme at WHO/HQ. There has been a shift from measles vaccine to measles/rubella vaccine. The increase in first dose vaccination has been gradual. The second dose vaccination is scaling up with one billion children vaccinated since 2000, as of July 2011. Reported measles cases have decreased by 60% and deaths have decreased by 74%, not the targeted 90% however good progress has been made. PAHO has been the only region to achieve elimination and WPRO is expected to be the next region to reach measles elimination with the target date being the end of 2012. There have been 15 large measles outbreaks between January 2011 and May 2012, and include the countries: France, DRC, Zambia, Somalia, Ukraine and Pakistan.

The challenges ahead are: India has had little decrease in measles since 2000; however they had a phased introduction of a second dose vaccine in 2010 as SIAs and in the routine programme. There has been a measles resurgence in Africa with a 4-fold increase in measles cases since 2008. There was a large outbreak in Burkina Faso in 2009, South Africa in 2010 and DRC in 2011. The older age groups have been involved in these outbreaks. The immunisation systems are still weak with sixty-seven countries having first dose vaccine coverage <90%. Financing is also a problem with countries not committing to 50% of the operational costs of follow-up SIAs.

A new Measles and Rubella Strategic Plan for 2012-2020 has recently been published. There is a unified vision of the core founding partners of the Measles and Rubella Initiative: The American Red Cross, UNICEF, CDC and WHO. The new elements include: Rubella and CRS elimination, activities to strengthen routine immunisation systems, outbreak preparedness and response, and research and development. The goals are to reduce mortality by 95% compared to 2000 and to achieve regional measles and rubella/CRS elimination goals by 2015, and by 2020, to achieve elimination in at least 5 WHO regions. Five key strategies are defined: (1) High vaccine coverage with 2 doses of measles and rubella containing vaccine. (2) Effective surveillance, monitoring and evaluation, (3) Outbreak preparedness and response, and case management. (4) Communication to build public confidence and demand for immunization. (5) Research and development.

In May 2012 a Global Vaccine Action Plan (GVAP) was formulated within the framework of the Decade of Vaccines and for measles and rubella is aligned with the MR Strategic Plan goals. Rubella is now seen as a “game changer”. The preferred approach is to begin with MR vaccine or MMR vaccine in a wide-age range vaccination campaign followed immediately with introduction of the vaccine into the routine programme. Countries introducing RCV should achieve and maintain immunization coverage of 80% or greater with RCV delivered through routine services and/or regular SIAs.

GAVI has pledged support for measles and rubella. This includes introducing rubella vaccine in an MR catch-up campaign to countries that have not previously been using rubella vaccine. This will be performance based funding based on first dose measles coverage and continued support for grants to
introduce MCV2 in routine schedule. There will also be support for measles follow-up SIAs in 6 large countries and support for measles outbreak response immunization.

PAHO is leading the way in the verification of elimination activities, WPRO held a 2nd Regional Verification Committee (RVC) meeting in 2012 and EURO plus EMRO have RVC’s formed and guidelines produced. There is a need to standardise the regional approaches. In order to achieve regional measles and rubella elimination goals by 2020, this requires quality indicators such as: reporting rate, adequate investigation, lab confirmation, viral detection and monitoring of viral excretion. In summary; funding no longer a major barrier, there is a shift from measles vaccine to measles and rubella vaccine in the GAVI eligible countries and regional verification of elimination is in progress.

An update on the Global Measles/Rubella laboratory network was provided by Mr David Featherstone, Global VPD Laboratory Network coordinator. The number of laboratories within the network totals 690 within 159 countries. From 2010 – 2011, 375,012 IgM tests were performed. In 2012 to 31st May 74,504 tests have been performed. Reporting timeliness (within 7 days) is as follows: AFR 59%, AMR 84%, EMR 98%, EUR 78%, SEAR 70% and WPR 79%. The WHO virus database currently has 13,312 measles entries and 1,302 rubella entries. The MeaNS database is going well with most labs now reporting sequences directly. From data reported to the databases, in 2012 there have been reports from 28 countries of 782 measles viruses, representing 5 Genotypes. For rubella viruses there are many gaps with 120 countries reporting lab confirmed IgM results in 2011 but only 13 reporting genotype information. Recent developments include a renewed interest in rubella. For example, SAGE (2011) recommends countries use their measles vaccination programme to introduce rubella vaccine. The WHO Rubella position paper (2011) recommends SIAs & routine immunization to interrupt transmission and prevent CRS. Field and laboratory surveillance for rubella should be fully integrated with measles. GAVI has provided funding for rubella vaccine introduction in 49 of 51 GAVI eligible countries. In Summary the LabNet has a strong capacity: >200,000 IgM tests, approximately 2,000 measles sequences and 330 rubella sequences are reported annually, the serological proficiency test pass rate is high, molecular surveillance is becoming increasingly important for monitoring progress of elimination and the verification process. Enhanced surveillance for molecular techniques has recently been field evaluated in AFR and SEAR; however there are still gaps, especially for rubella molecular surveillance. Financial support for the LabNet continues to be tenuous and a new coordinator will be needed by the end of September who is expected to maintain the achievements, address the challenges and support the continued development of the network.

2. Regional LabNet updates; progress and challenges

AFR-Dr Annick Dosseh & Dr Charles Byabamazima

Measles elimination for the African region has been set for 2020. Pre-elimination targets have been introduced with the status as of end of 2011 as follows. 7/46 countries on track (Botswana, Burkina Faso, Ghana, Malawi, Mauritius, Seychelles and Swaziland), 25/46 countries are missing one target, 14/46 countries are missing 2 or more targets. Co-ordination of network, including testing algorithm, procurement, quality control & data reporting has not changed. Data reporting is done on weekly basis. Monitoring of laboratory performance is done by confirmatory testing and annual proficiency testing. There are 44 labs in the Region including 3 measles RRLs. Capacity building is continuing
with most training performed in 2012 on Yellow fever testing. In May 2012 there was measles phylology training for one person in the Uganda laboratory. Timeliness (within 14 days of specimen received) in providing confirmatory testing is variable between the RRLs: Cote d’Ivoire 68% (2011) and 65% (2012); Uganda 64% (2011), and 100% (2012) and 5th Africa 100% (2011), and 99% (2012). Forty-one laboratories participated in the proficiency testing in 2010; all achieved a score of ≥90%. Thirty-six labs participated in 2011, but one achieved a score of <90%. Some laboratory results are still pending. The genotypes circulating in the region from 2005-2012 are: B3, B2, D4, D2 and D10. The challenges for the regional LabNet include: shortage of measles/rubella kits, mainly due to funding issues, especially with Nigeria which has a huge kit supply need; no data reporting: Ethiopia (since July 2012), Mauritania (since April 2010). Country clearances have sometimes not been obtained for accreditation visits therefore some have been postponed. There has been an interruption of sequencing activities at NICD so a number of key isolates are pending; there is also a funding gap for lab activities as well as competing activities.

EMR-Dr Hinda Ahmed

The major challenge in the region has been the political uncertainty and conflict occurring in several countries of the countries. The measles elimination target has been postponed until 2015. The strategy is to achieve high population immunity with the first dose coverage being >95% and also >95% for the second dose. In 2011 the coverage was 84.8%. Routine second dose and/or periodic follow-up SIAs should continue until the high routine coverage is achieved for MCV1 and MCV2. All 23 countries in EMR have the capacity to perform serology and twelve of them are able to perform serology plus virus detection. Another six laboratories are able to perform serology, virus detection and sequencing. Laboratory performance is measured by the Global Proficiency test on serum. In 2011 all countries participated except Qatar, S. Arabia & Yemen. All of those participated received a 100% score. Serum validation: All countries except Djibouti, Kuwait and UAE sent samples for serum validation and received good concordant results. Virus detection and Sequencing: All countries with measles cases sent or genotyped except Djibouti, Lebanon and UAE. PT on FTA filter paper: For RNA extraction, RT-PCR, Real time RT-PCR and sequencing were provided to 10 countries and the results evaluated by global measles and rubella lab CDC Atlanta. Since 2007 several training workshops were undertaken for molecular techniques, sequencing techniques, sequence analysis and the reporting of genotypes to the MeaNS database... For 2011-2012 there were outbreaks in Afghanistan, Pakistan, Somalia, Sudan and Yemen due to low measles vaccination coverage and delay of follow-up campaigns for financial constraints. In 2011 there was a large rubella outbreak in Tunisia. The measles genotypes identified in 2011-2012 were B3, D4, D8, D9 and H1. The rubella genotypes were 1E, 1G, 1j and 2B these were identified in nine countries. Challenges include funding for immunisation especially follow-up campaigns; seven countries are in need of a follow-up campaign. Security is a major problem and is rapidly deteriorating. Also the sustainability of LabNet surveillance is a problem as there is insufficient support of M/R LabNet activities (provision of reagents and equipment) by some member states. The rubella outbreak in Tunisia continues and needs to be investigated. In summary, there has been progress in measles elimination. Nine countries are very close to measles elimination and vaccine coverage has improved in priority countries. The future activities include strengthening the routine immunisation program, improving surveillance and conducting workshops to strengthen measles and rubella elimination performance indicators.
**EUR-Dr Mick Mulders**

The European Region has a measles and rubella/CRS elimination goal by 2015. Eastern Europe has good coverage, Western Europe has not reported its coverage for some areas and the others have low coverage. There have been measles outbreaks in Bulgaria in 2010 and in France in 2011 and Ukraine in 2012. The 5-19 age group have the higher incidence of measles. There has been a large outbreak in Romania which is ongoing and mixed, both measles and rubella. The laboratories have an enhanced role in approaching elimination, this includes: routine investigation of all suspect measles and rubella cases (serology), timely reporting, close collaboration with MR program, conducting seroprevalence surveys and genotyping. All national and sub-national laboratories that participated in the accreditation review in 2011 are (provisionally) accredited (n=67). Proficiency testing PT01106, NL and BIH did not participate and France only tested for measles. All laboratories passed for measles (68/70, 100% score) and rubella (66/6, 100% score). Many laboratories do not send enough samples or sample volume for confirmatory testing and they often send samples from cases without clinical background and from screening programs. Confirmatory testing score (passing score ≥90%), for National Laboratories only, was 41/46 100% (all passed) for measles and 34/46 100% (all passed) for rubella. In 2011 and 2012 D4 remains the predominant endemic genotype with scattered clusters of B3, D8 and D9 most set off by importation events. The meetings held in the region include the annual RRL meeting, a joint meeting RUS/NIS, a Molecular workshop (RIVM) and individual training for Turkey and Kazakhstan. The challenges are: To establish discard rate (the number of negatives tested), timeliness of sample collection, timeliness and completeness of reporting data to WHO, genotyping baseline (particularly rubella), financial sustainability of LabNet, continuing outbreaks, pockets of susceptibles (un-/ under immunized). The future plans are: Finalize pilot MR-LDMS and connect laboratories 2012-2013, conduct annual meeting for RUS and NIS 2013, meeting for EUR laboratories (English) early 2013, meeting/workshop for Balkan late 2012, biosafety training as part of annual meeting, conduct training workshop molecular methods (2012-2013), introduction of LumineX/array technology in EUR LabNet, develop guidelines on seroprevalence studies as part of guidelines for verification of elimination and roll out molecular EQA.

**WPR-Dr Youngmee Jee**

The elimination goal for the Western Pacific Region is 2012. Measles incidence for 2011 was 11.8% and 4.8% for 2012. The coverage rate for MCV1 is low for the Philippines and the Solomon Islands (70 – 79%) and for Laos, PNG and Samoa (50-69%). China had a measles vaccination campaign in 2010 and the number of measles cases has decreased by 75%. Forty-seven out of 48 fully functional network laboratories including 31 provincial labs in China are accredited as of June 2012. After the SIA in several countries in 2010-2011, measles incidence dramatically decreased in 2011-2012 (e.g. China, Vietnam). Rubella positive cases are increasingly detected in the region: Vietnam, Cambodia, PNG, Fiji and other PICs in 2011. The proficiency panel for 2011 was distributed during the LabNet meeting in September (46 sets), 20 additional sets of PT samples was provided to Japan, Viet Nam and Malaysia. Confirmatory testing is performed at least once a year, but preferably twice a year from 2010. Concordance rates of confirmatory testing were ≥90% (100% for many labs). Recent genotyping data is available in most countries. Testing of samples from CRS surveillance is on-going in Viet Nam and planned for PNG. The challenges: confirmation of measles cases in non-NML labs in some countries (e.g. Japan, Singapore, Vietnam, Australia, and New Zealand), quality assurance of non-NML testing is needed, consideration of more labs for participation in WHO PT programme: Vietnam, Malaysia and Japan in 2011-2012, capacity of some NMLs to conduct virus isolation and
molecular detection, confirmation of measles cases in non-NML labs in some countries (e.g. Japan, Singapore, Vietnam, Australia, and New Zealand), high workload for Hong Kong RRL: 1) confirmatory testing 2) most additional genotyping work performed only in HK RRL. Incomplete clinical/epidemiological information for result interpretation and further testing. Plans for the region include: further strengthening of strain surveillance for measles from all chains of transmission, both sporadic and outbreaks (and rubella). WPRO MR Bulletin provides monthly updates. Optimal use of DBS (Cambodia, PNG, Fiji and Philippines) and oral fluid collection (Philippines, New Zealand).

More labs for WHO measles and rubella serology PT: 4 for Vietnam, 2 for Malaysia. Follow up hands-on training in Hong Kong in Oct 29-Nov 3 2012.

AMR-Dr Gloria Rey

AMR has achieved elimination of measles and rubella. There are 21 National laboratories, 2 RRLs, 1 Global specialized laboratory. Surveillance indicators are improving with 90% of laboratories reporting in <4 days. The Region now faces the challenge of timely detection of imported virus. Most countries have documented importation of measles with genotype identification. The genotypes identified in 2011 were D4, D8, D9, G3, H1 and B3. D4 was the most common genotype imported. The Region of the Americas has advanced in the verification process of the elimination of measles, rubella and CRS. The Lab Network continues to meet the WHO/PAHO criteria for accreditation and to conduct proficiency tests. Most countries have documented interruption of previous endemic measles and rubella genotypes. Canada document importation of rubella genotype 2B in a CRS case. The AMR has increased the collection of second serum samples and samples for virus identification to improve the classification of the cases; at the same time and taking in mind the context of low incidence the introduction on new techniques into the Regional LabNet is going on (IgG, avidity test, real time RT-PCR and sequencing). The challenges that the region faces are: Maintaining commitment for adequate specimens to perform genotyping, maintaining links between epidemiology, immunisation and the laboratory teams, continue the implementing of new molecular techniques, introduction of a PT program for molecular techniques. Summary: Lack of endemic measles and rubella. The molecular epi data collected between 2000 and 2011 helped to document the end of circulation of endemic measles and rubella genotypes in the Americas. Financial support needs to be maintained. Communication between epidemiology, immunisation and the laboratory teams to maintain quality surveillance.

SEAR-Dr Jagadish Deshpande

The SEAR region has set an elimination goal of 2020. The strategy is to improve and sustain vaccine coverage and provide a second vaccine dose through catch-up campaigns and also to improve surveillance, 2012 is to be the year of “intensification” of routine immunisation. In India 17 states have introduced MCV2, MR vaccine was introduced in March 2012. Case based surveillance has been implemented. The testing of samples within 7 days needs to be improved. From 2007 to present time the measles genotypes identified were D4, D5, D7, D8, D9, G2, G3 and H1. The rubella genotypes were 1E and 2B. Progress made in the region includes the expansion of 8 laboratories to 24. All countries have access to virus detection and isolation (15 laboratories in total). Eight labs participated in the most recent PT panel. The submission of sequence and genotyping is improving. A decision needs to be made on the use of oral fluid (OF) in the region. By using OF virus detection as
well as serology can be performed. The challenges include introducing new laboratories: parallel testing is occurring at some labs and this increases the need for resources. Urine specimens are being collected at some labs for virus isolation. The priorities are to update accreditation of laboratories, organise laboratory training and virologists meeting, independent reporting by new labs after proficiency confirmation, identify new Measles/ Rubella network labs in India (2), Indonesia (2) and Nepal (1), improve virus isolation sample collection and encourage virus and genotyping.

Session 3: Enhancing Surveillance for Measles and Rubella

Dr Charles Byabamazima and Dr Annick Dosseh presented a review of oral fluid surveillance in Africa. In 2009 a pilot study was performed to determine field feasibility of using OF. The participating countries were Benin, Cote D’Ivoire, Kenya, Malawi and Zimbabwe. The process consisted of field training, serum and OF were collected and shipped together. The serum samples were tested with Siemens kits and the OF samples tested with Microimmune kits in batches (to save kits).

The results in the field were that samples were easy to collect with no added shipping cost. Instructions on Oracle packets were considered to be vague, leading to collection of saliva (thorough orientation essential) and the oracle devices were not available in health units for routine tests. Oral fluid samples were received from sites of the 5 countries: 3 stopped in 2011(Kenya, Malawi & Zimbabwe) and 2 continued into 2012 (Benin & Cote D’Ivoire). The AFRO algorithm was followed (test all for measles and test the negatives for rubella), thus fewer rubella tests were performed. ELISA testing on serum was performed with the Siemens kit and OF with Microimmune kits. One site (Malawi) tested OF only for measles. The OF showed good correlation with the serum. The sensitivity for OF confirming measles cases compared very well with serum > 90% (except Malawi). The sensitivity for Rubella was 79% for Cote d’Ivoire and 83% for Kenya. The next steps will be to discuss results and agree on application of OF sampling in routine surveillance. There are 18 potential countries to be involved. Surveillance teams need to be informed of pilot test results and plan for training on the use of oracle devices and Microimmune kits then implement a schedule. There is a need to mobilise financial resources for scaling up. Virus detection using OF will be performed in selected countries e.g. Rwanda & Tanzania. Overall sensitivity would most likely improve if virus detection is added.

Dr Rob Van Binnendijk gave an update on new technologies for monitoring susceptibility and performing surveillance using multi-antigen IgG and IgM detection. The main objectives are to standardise testing for evaluation of immune status and to enhance the compliance of IgG/IgM surveillance and patient evaluation. Also to provide a rapid diagnosis of infection during an outbreak. The two methods are Luminex technology and protein microarray. The Luminex technology allows a high sample throughput, is very specific with low limit quantitation. The test allows sample and antigen saving and reduces hands on time. The protein microarray technology is performed on nitrocellulose or glass slides. Low antigen input is required; it is flexible and allows a differential diagnosis. The assistance these technologies can give to the WHO lab network are: independent testing of IgG/IgM levels (at high throughput), avidity testing and preparation of reagents (beads & slides). Analysis of non-invasive samples such as DBS and OF needs to be performed.
An update on POC assays using OF and serum was given by Dr Dhan Samuel. The POCT is based on lateral flow for detection of measles IgM in oral fluid and serum samples. It was evaluated using samples collected from the UK and Zimbabwe. The sensitivity was 90% and the specificity 93% for both serum and oral fluid. Measles nucleic acid can be extracted from the POCT strips. The limitations of the test were that it was cheap to manufacturer but only small batches could be made at one time. The strips needed to be handled which could lead to contamination and serum separation was required. Pipettes were still required and the readings were subjective. These limitations made it difficult to perform as a field test. A manufacturer (Forsite) was approached to produce test strips on a large scale and encase them in cassettes plus incorporate a blood separation pad to allow capillary blood to be tested. The specifications were that the manufactured POCTs must have the same performance as the in-house produced test. The first prototype resulted in background staining with all Forsite POCTs. The second prototype with blood separation pad and incorporating additional surfactant in the cassette format produced by Forsite had reasonable sensitivity but very poor specificity compared to the in-house POCT. For the third prototype Forsite agreed to “dip” block the membranes in protein solutions for one minute and drip-dry before assembling into POCT cassettes. Twenty prototype cassettes with various blocking solutions were tested. The evaluation showed that blocking might resolve the non-specific reactivity issues. The POCT readers remove some of the subjectivity. The prototype 4, using 0.25% and 0.5% casein will be produced using the manufactures reel to reel equipment. If the in-house laboratory evaluations look promising it could be evaluated in field sites. Investigation of miniature disposable pastettes to deliver accurately small volumes, such as 20ul and 100ul is on-going plus improvements to oral fluid collection device to enable testing at the point of collection. The development of these features will make the POCT a real field test useful for surveillance.

Session 4: Quality Assurance and Standardization

Ms Jennie Leydon gave a summary of the measles/rubella IgM proficiency test for panel 01106.

Two-hundred and eleven laboratories participated in the panel testing. All but one laboratory scored >90% for measles and all laboratories scored >90% for rubella (a pass for the proficiency test. However a number of laboratories had results that differed from the optical densities that the majority of laboratories reported. An investigation into the testing procedure of these laboratories should be made. Seventy-nine percent of laboratories returned their results to VIDRL within 14 days and 94% tested the panel within 14 days of receiving it.

Dr Paul Rota discussed the evaluation and plans for implementation of a molecular PT programme. The validated protocols and kits have been established. It is recommended that a take home practice panel should be included as follow up for all intercountry training courses focusing on molecular methods. All RRLs and selected NLs performing measles/rubella molecular testing should participate in the global molecular PT program. Methods/protocols to be assessed by proficiency testing are RNA extraction, detection of measles or rubella RNA by endpoint RT-PCR (agarose gel electrophoresis), generation of recommended sequencing templates for measles and rubella by RT-PCR, sequencing to support standard genotype classification of measles and rubella, real time RT-PCR to detect RNA from measles or rubella virus, Sequence analysis to determine the genotype of measles and rubella using the protocols and reference strains recommended by WHO and sequence submission to MeaNS (eventually RubeNS) and genotype submission to the WHO Genotype Database. Initially molecular PT panels will be distributed to the laboratories one time per year. The frequency may decrease if laboratories consistently demonstrate high performance. Panels will also continue to be distributed during intercountry training workshops. As per recommendations of the Global Meeting of 2011, the FTA panels will be produced by CDC and sent to Regional Laboratory
Coordinators for distribution. A standard reporting form will be developed for returning the results to CDC and the Regional Laboratory Coordinator. Results must be sent by e-mail. Participants must follow the reporting protocol very carefully as a successful reporting includes both summary information as well as the raw data.

Dr Joe Icenogle reported on the CDC standard kits for rubella virus detection. The kits produced contain primers, probes, control templates, and instructions. The kits do not contain materials such as enzymes and nucleotides. The CDC assay can detect as low as 10-30 copies of viral RNA with results in about 4 hours, it contains reagents for performing a qualitative assay using TaqMan® chemistry. Two RNA controls are included a high control and a low control. The RNA controls also contain human RNA to serve as a template for the RNAse P reference gene. Test samples are run in triplicate with rubella primers and in a single well with RNAse P primers. A rubella practice panel has been produced using FTA elute cards. The rubella diagnostic Real-time RT-PCR Kit and Rubella Virus Genotyping Kit have been improved in the past 12 months. Stability during storage has been evaluated. The FTA controls are still good for practice panels after 10 months storage (4°C with desiccant). The RT-PCR kit is stable for 17 months in storage (4°C) and the genotyping kit is stable for 21 months. The Real-time RT-PCR and Genotyping kit works on the LC480 platform, other platforms will be evaluated as part of upcoming EUR course.

Session 5: Challenges of meeting Elimination Criteria for measles and rubella and meeting LabNet needs for monitoring new rubella control strategies.

Mr David Featherstone reviewed the current measles and rubella assays to check that they met the needs of the programme. The majority of LabNet uses the Siemens commercial IgM assay for measles and rubella. When Siemens took over Dade Behring in 2008 a number of issues with the kits were reported, the main issue being with the positive control (P/P) showing declining value with time. Countries with no or very low incidence may repeat weak positives and equivocals numerous times with variable results. Using the results reported in the global proficiency test (01106) a review of the assay was performed. The assay batch and time of testing was recorded. Identical samples tested in more than 200 labs with > 144 using Siemens kits. Six different lot numbers were in use for measles and 7 different lot numbers were in use for rubella. Summarizing the results the positive control P/P values were relatively consistent, all except 2 values were within the upper and lower margins. Overall the PT results were consistent; some issues with results were possibly laboratory specific.

Data from Brazil labs was also analysed, one lab retested samples with equivocal results and another study performed a comparison of previous results and repeat testing of 3-4 replicates in the same plate. The conclusions drawn from this study were that retesting of samples with ODs close to cut off will give qualitative results which may fluctuate and an edge effect was seen in multi-replicate testing. Labs observing unexpected results or having concerns about assay consistency should carefully document their results and share with LabNet coordinators and reference labs.

Dr Gloria Rey discussed the challenges of meeting elimination criteria for measles and rubella as experienced by the Americas. One of the indicators of the quality of surveillance relies on the laboratory for confirmation of suspected cases. Viral detection and genotype information should be available from at least one specimen of each chain of transmission (outbreak). In order for laboratory activities to continue to verify elimination the main factors to consider are: Quality control, case...
classification and laboratory testing for sporadic cases, molecular epidemiology (genetic baseline established), laboratory surveillance for CRS and monitoring of virus shedding, appropriate samples collected, maintenance of adequate resources and virus containment. One example of monitoring of virus shedding was showed after the last rubella outbreak in Argentina, where the surveillance system detected 12 CRS cases, between 2008-2009, and the excretion of genotype 2B was did for several months.

Dr Hinda Ahmed reported on the Eastern Mediterranean Region LabNet for surveillance of rubella/CRS elimination. Not all countries in the region are using rubella vaccine; there are seven GAVI eligible countries. Oman has set an elimination goal for rubella for 2010. Seven countries (Bahrain, Egypt, Iraq, Jordan, Qatar, KSA and Syria) have an elimination goal of 2015. Kuwait has Rubella elimination goal of 2020. Iran, UAE and Libya have no definite date. Three countries (Lebanon, Palestine and Tunisia) have no definite date for CRS elimination. There are 8 countries with no vaccine and no target (Afghanistan, Djibouti, Morocco, Pakistan, Sudan, South Sudan and Yemen). All labs in LabNet test suspected measles cases that are negative for rubella IgM. The laboratories have the capacity to test for IgM antibody, perform virus isolation or detection of viral RNA by RT-PCR or Real Time RT-PCR, sequencing and genotyping. Rubella testing is only performed in 23 countries, 14 countries test in parallel with measles and 9 countries test measles negatives only. In 2011 in Tunisia a rubella outbreak resulted in 994 lab confirmed cases. To prevent congenital rubella, following the recommendations of a WHO consultant in 2004, the following strategy was set. Use only rubella containing vaccine for vaccination of girls at 12 yrs in 2005, a vaccination campaign for girls aged 13-18 yrs in 2005, vaccination of all postpartum primiparas with no evidence of immunity; and multiparas with negative serology during pregnancy and introduction of sentinel surveillance of congenital rubella in three centers. Considering the epidemiological situation and the outbreak in Tunisia, it is recommended to initiate as soon as possible: routine vaccination of boys also at the age of 12 years, two doses routine vaccination MR for children 15 months and 6 years plus a catch-up campaign MR for children aged 7 to 11 years. In addition it is recommended to: strengthen immunization postpartum, strengthen the CRS surveillance, analyse the characteristics of the epidemic and options for optimizing the immunization schedule for measles and rubella. There is also the opportunity using GAVI support for MR catch-up campaign 9m-14yrs in those countries eligible. The challenges to be met are: Source of funds for sharing operational cost of the catch-up MR campaign, source of funds for introduction of the routine vaccination, source of funds for follow-up SIAs and availability of local data to document the need for rubella vaccine introduction.

Dr Marilda Siqueira discussed Lab support for surveillance during approach to elimination in Brazil. The guidelines for investigation of suspected measles/rubella in Brazil include: Integrated measles/rubella surveillance, mandatory notification of suspected cases (i.e. fever and rash), collection of serum specimens at first contact with patient, collection of a specimen for viral isolation when there is known exposure or high index of suspicion and collection of additional specimens for IgM positive patients. The last confirmed measles case linked to endemic measles virus was in 2000. The last confirmed rubella case linked to endemic rubella virus was 2008 and the last confirmed CRS case linked to endemic rubella virus transmission was 2009.

The key elements for laboratory surveillance in low incidence settings are: Case classification and interpretation of results require consideration of the specific situation, sample timing, and the
appropriate use of multiple, validated diagnostic tests. Cases in recently vaccinated persons
or sporadic IgM+ that are suspected to be false positive continue to be difficult to classify. Measles,
rubella and CRS cases need careful reviewing. IgM positive or inconclusive results should be
followed up with a second sample, if possible. IgG testing should also be performed as an extra aid in
interpretation of a positive IgM on a single sample. Other IgM tests should be performed as a
differential diagnosis (Dengue, HHV6, and Parvo B19) but should be carefully interpreted, especially
in a second sample is not available to access IgG results. Virus detection on appropriate samples
should be considered. Some protocols were developed: 1) The protocol for measles or rubella
identification in Dengue or Parvo B19 outbreaks needs to improve in some States; and 2) A protocol
for pregnant women with rash diseases that is established in all States. The challenges are; being alert
to MR occurrence in the world as a possibility of importation, maintaining political will in the absence
of disease and the continual need for staff training.

**Dr Kevin Brown** discussed the lab support for surveillance in the UK. The rubella immunization
strategy in 1970 was the immunisation of females of child bearing age. In 1988 MMR vaccination at
15 months of age was introduced and a catch-up campaign was performed in 1994. In 1996 second
dose MMR (3yr 4mths – 5yr) was introduced and another catch-up campaign was performed in 2008.
The last rubella outbreak was in 1996 and was mostly in adult males. CRS is now rare; there have
been 17 congenital rubella cases reported since 1997. There were 12 rubella cases in 2010, 6 in 2011
and 45 cases in 2012. The cases in 2012 were in clusters in boarding schools and associated with an
importation from Romania.

There is routine antenatal screening for pregnant women which includes screening for hepatitis B,
HIV, syphilis infection and rubella susceptibility. The National uptake of antenatal screening for both
syphilis and rubella susceptibility increased from 94% in 2005 to 97% in 2010. Testing is by ELISA,
and <10IU/ml is reported as susceptible (based on data from 1995). The national rubella susceptibility
rate in screened pregnant women was 2.6% in 2005 and 4.5% in 2010. Different rubella assays give
different results, so the susceptible level differs from assay to assay. The diagnosis of rubella should
be confirmed by PCR if patient is IgG negative and rash onset less than 7 days. Both capture and
indirect IgM assays are used to confirm a positive IgM and if IgG positive and an avidity assay can
also be used. As part of enhanced surveillance, a questionnaire is sent out, a register for all rubella
infections in pregnancy is kept and samples are requested at delivery and up until one year of age.
There has been <1:100 clinically suspected cases confirmed since 1999, an over estimation of
incidence is based on notifications. The WHO and EU clinical case definition lacks specificity and
emphasizes the importance of laboratory confirmation during elimination phase. Enhanced
surveillance utilizing oral fluid testing is widely accepted.

**Dr Joe Icenogle** discussed the lab support for surveillance during long term maintenance of
elimination in the USA. Rubella was eliminated in 2004. Annually between 2004 and 2011 the
median number of rubella cases was 10 (range 3-18), eighty-seven percent of cases were unvaccinated
or the vaccination status was unknown. During 2004-2011, in total, 76 cases were reported from 58
counties, with no county reporting more than 4 cases for the entire period. Due to the extremely low
prevalence of rubella, false IgM positives are expected to occur almost entirely in vaccinated
individuals. Differentiation between a false IgM positive and a rubella case is through additional
laboratory tests i.e.: avidity testing, repeat IgM testing and IgG testing.

From 1996 to 2011 the genotypes identified were 1B, 1C, 1E, 1G, 2B and 1j. From 2006 to 2011 there
were no clusters observed in suspect cases referred to CDC. In 2011 it was concluded that elimination
has been achieved and maintained. It was recommended that the WHO measles and rubella laboratory
network should maintain information on efforts to maintain elimination of rubella and CRS (as well as measles) in countries where these diseases have been eliminated, noting the surveillance, investigation, and response activities needed to sustain elimination of each disease.

Dr David Brown discussed the role of seroepidemiology in the elimination phase of measles and rubella. Seroepidemiology can give a direct measure of the performance of the vaccine program. The two approaches for collection of serum banks are: collect residual serum from laboratories or collect population base samples. The residual samples are a cheaper option but may not be representative and no vaccine history would be available. The population collection is a more expensive option and may have a bias due to refusals. Vaccination data would be able to be collected and groups of interest could be oversampled.

The measurement of a protective titre needs to be considered, in 1990 a study concluded that titre of 120 mIU/ml for measles was protective. For rubella, the 1960s saw the validation of HI test showing non-specificity at low titre due to lipo proteins in serum giving no specific inhibition of haemagglutination (15 IU / ml). The introduction of EIA enabled reliable detection of levels of antibody now set at 10 IU / ml, which is protective in vast majority of cases. With good Rubella control antibody levels are still dropping, many now <10IU/ml, with current EIA’s cut off nearer 4 IU/ml.

Conducting seroprevalence studies is valuable to the programme, but it involves significant time and money. It may not be feasible for all countries. Therefore, it is recommended to use all sources of serological data. The recommendation would be to develop a guidance document for conduct and analysis of seroprevalence studies for measles and rubella. Compare performance of available EIAs and newer platforms (Luminex etc.) for current levels of population antibody.

Seroepidemiology of MMR in Germany was presented by Dr Annette Mankertz. A survey of children and adolescents nationwide was conducted from 5/2003 to 5/2006, 17,641 girls and boys 0 to 17 years were enrolled. A questionnaire was sent to all parents of 0-17 year olds and to adolescents 11-17 years. A computer based interview and medical examination was performed, a copy was made of vaccination cards and blood and urine was collected. IgG testing was performed for MMR and correlated to information gathered. There were 10% less seropositive than those vaccinated. For measles 8% of early measles vaccinated children did not develop a detectable titre, despite a later administered MCV2. There were several twice MMR-vaccinated individuals that were seronegative by ELISA, 25 of these were tested for measles by FRNT and all were positive. Due to the lower sensitivity of the ELISA test compared to FRNT, there is an overestimation of measles seronegatives using an ELISA-based approach. There was increased MMR seronegativity in 1-2 year olds due to delayed first MMR vaccination and waning immunity was highest among adolescents. Children not presenting vaccination cards are more often seronegative. Seronegativity for measles is associated with vaccination at a very young age. Children born in Germany whose parents had a migration background were less often seronegative than their German contemporaries, while children born in foreign countries had a higher risk of being seronegative. The conclusions drawn from the serosurvey is to further increase the two-dose vaccination coverage and improve the timeliness of MCV1 and MCV2. Catch up vaccination campaigns for adolescents and immigrants are recommended as well as convincing high educated groups of vaccine benefits.

Dr Joe Icenogle presented an overview of current utilization of tools for genetic baseline and molecular surveillance. In 2010 there were 130 countries using rubella vaccine. There has been a global measles and rubella strategic plan since 2011. SAGE recommends that countries should take
the opportunity of the two dose measles vaccine strategy to use MR or MMR vaccine. GAVI will support eligible countries to use the rubella vaccine, in combination with a measles vaccine, as part of catch-up campaigns targeting children between the ages of 9 months and 14 years 11 months. There will have been, by the end of 2012, at least 5 intercountry laboratory training workshops on measles and rubella which included Real-Time RT-PCR for rubella virus (in AMR, EMR, EUR, and WPR) and a number of intra-country training activities which included Real-Time RT-PCR for rubella virus. In next six years about 50 GAVI-rubella eligible countries are expected to introduce rubella containing vaccine. CDC has been facilitating rubella genetic baseline determinations in 3 GAVI countries: Uganda, Cote d’Ivoire and India. Uganda has established rubella virus isolation and detection techniques in their laboratory and have obtained at least 15 virus sequences covering a period from 2003-2012. All were genotype 1G, establishing that 1G is an endemic genotype in Uganda. Cote d’Ivoire sent a panel of 49 rubella IgM positive sera to CDC for genotyping, 8 genotypes were obtained and all were genotype 1G. 1G is likely the endemic genotype in Cote d’Ivoire but additional India’s lab in Pune has submitted 4 rubella virus 739 nt sequences from 2007-2009 to GenBank. All are genotype 2B. The recommendation would be that given the certainty of introduction of rubella containing vaccine into many countries that have not yet established a rubella virus baseline genetic, such countries working with RRLs should, at least, develop and implement a plan for collection and storage of rubella specimens for genetic baseline determinations.

Additional tests for classifying cases in low incidence settings was discussed by Dr Kevin Brown. The avidity assay is usually performed using serum and an indirect ELISA format and in a primary infection remains low for 3-4 months. A comparison was performed to see if avidity testing could be performed using oral fluid. The conclusions were that avidity can be used for both serum and OF samples and it correlates well with IgM results. High avidity antibody was detected in those who have previously been vaccinated. Avidity should be interpreted along with the measles IgG result, the PCR and the vaccine history, if it is available.

**Session 6: Measles Surveillance: Tracing measles transmission pathways, identifying gaps and proposals for improving surveillance performance.**

**Ms Sheilagh Smit** updated the genotype data for the African region. The DRC (2010-2012) sent 80 specimens, 47 were PCR-positive (59%). The genotypes were B2, B3 RWA-like and B3 ESA-like. Cote d’Ivoire sent 22 specimens, 11 were PCR positive. They were genotype B3 from Nigeria and Ghana.

**Dr Josephine Bwogi** presented the molecular surveillance from Uganda. The measles vaccine coverage was 71% in 2011, in 2012 there was a mass measles vaccination campaign; this was combined with polio and administration of albendazole. From 2003- 2012, B3 was the measles genotype circulating. In 2011, 28/112 (16%) districts had at least one measles case reported; there were measles outbreaks in 8 districts. Genotype B3 was detected in Tanzania and Rwanda, and in Uganda, D4 and B3. Historical samples for rubella from 2003, 2007, 2008, 2009 and 2010 detected genotype 1G. The challenges for Uganda include a shortage of RNA extraction kits and PCR enzyme kits and competition for the use of a sequencing machine. In conclusion; B3 and D4 measles genotypes are circulating in Uganda, the D4 is most likely an importation and the 1G rubella genotype is also circulating in Uganda.
Dr Henda Triki gave an update for the Eastern Mediterranean Region. The region has 23 countries, with 1 NML per country, 18 laboratories have the capacity for virus detection and 6 laboratories can perform sequencing. From 2000-2012 the major genotypes detected were D4 (47%) and B3 (38%), other genotypes detected were D5, D8, D9, H1, A and C2. More efforts are also needed to improve timeliness of genotype identification, sharing sequence data and reporting sequences to WHO genotype database and MeaNS.

Mr Sohail Zaidi gave an update on Pakistan and Afghanistan from 2007 to date. In Pakistan D4 was the only genotype identified from 2007 to 2010. In 2011 genotypes D4, B3 and H1 were identified and for 2012 D4 and B3. In Afghanistan in 2008 genotype D4 was identified, 2011 genotypes D4 an H1 and in 2012 genotype B3. The main challenges identified were: limited numbers of Lab Staff, virus isolation has not been successful, non-timely provision of kits and reagents and the optimum time frame for sample collection of oral fluid needs to be identified.

Ms Patcha Incomserb presented the data for the South East Asian region. There are 11 countries in the region. From January to 31st May 2012 genotype D9 was found in Myanmar and Thailand and genotype D8 in Nepal and Thailand. D8 has become the predominant genotype in SEAR. From 1993 to 2008 D5 was predominant, D9 from 2008-2011 and D8 2011-2012. Rubella genotypes in the region are 1E and 2B.

Dr Sabine Santibanez presented the data from Germany; from August 2011 to May 2012 the genotypes identified are B3, D4 (predominant), D8 and D9. Currently there is an outbreak in Poland in a Roma community that emigrated from Romania. Measles activity is continuously high in Romania and is declining in Italy. There have been variants of the D4 genotype, the majority from Europe.

Dr Judith Huebschen presented measles data for the countries supported by the Luxembourg RRL. Portugal had 1 case of D4-Manchester in Lisbon with an epi-link to London, UK, and 1 case of B3 with an epi-link to Angola. France has an ongoing outbreak with mainly D4-Manchester type viruses, the genotype A strains were vaccine associated and sporadic cases or small clusters of some other genotypes, sometimes with epi-links to abroad (e.g. B3 from Cameroon) were found. The Netherlands had a few sporadic cases in April 2012 in unvaccinated people; D4 with epi link to Spain and D8 with epi link to Thailand. In Luxembourg there was one case of vaccine associated measles. Israel had B3 importations from Angola and Eritrea, a D8 importation from India and an ongoing outbreak in the Tel Aviv region. Reasons for the limited amount of molecular data in the RRL Luxembourg constituency comprise the lack of possibilities to genotype in some countries, problems with funding (e.g. Spain), a big workload, lack of appropriate specimens and of awareness of the importance of molecular data in some countries with low case numbers.

Dr Kevin Brown presented the UK data. There was a peak of measles activity in May 2011 and again in 2012. The confirmed measles genotypes from 2010 to 2012 were H1, G3, D9, B8, D4, B3 and A. In 2012 the genotypes found were D9, D8, D4, B3 and A with the predominant genotype B3 mainly in Nth West England. Checking the sequence diversity of B3 genotype, they were mostly identical and the D4s were mainly the Manchester strain. In reference to the strain bank activities, the measles strain bank has 120 different isolates; 15 different genotypes: A, B1, B2, B3, C2, D4, D5, D6, D7, D8, D9, D10, G2, and H1. There were not isolates reported in 2011/2 and no ‘D4 since 2007’. It is requested that if you culture, please submit strains, or indicate in MeaNS that you have a culture available. The Rubella strain bank has 29 different isolates 1a, 1B, 1C, 1D, 1E, 1F, 1G, 1h, 2A, 2B.
Dr Sergey Shulga reported the genotypes in Russia and neighbouring countries. In 2011 D4 was the predominant genotype. From 2011 to 2012 there were small outbreaks of measles reported these were mostly imported cases. In 2010 there was an increased incidence of measles with D4 being the predominant genotype which became endemic in the region. In summary the predominant transmission of 2 clusters of D4 genotype has been observed in the CIS region. The “D4 Enfield 2007” strains were mainly imported from Western Europe with, ongoing transmission in Ukraine. “D4 Iran 2010” strains were imported into the region in 2010, caused outbreaks in Uzbekistan, Kazakhstan, Kyrgyzstan and Russia with ongoing transmission. Genotypes D8, B3, G3 were imported mainly from Europe but not from endemic settings directly.

Dr Yan Zhang reported that there has been a decrease in measles cases in China. There has been a 54% decrease in 2012 compared to the same period in 2011. Infants and young adults account for more than half of the measles cases. Most of cases had less than 2 doses of vaccine or unknown vaccination history, especially the young infants and adults. The measles cases were concentrated in a few provinces and most of the cases were sporadic. The NML performs the genotyping and reports back to the provincial labs and to WHO. For 2011, there was a total of 294 isolates from 25/31 Provinces, the genotypes were: H1a (288), A-VL (3), D11 (3). For 2012 there was a total of 179 isolates from16 provinces, the genotypes were: H1a (172), A-VL (1), D9 (6). The D9 cases in 2012 showed 99.8-100% homology.

Dr Paul Rota updated the Americas data. There were 1310 cases of measles in the Americas in 2011; the genotypes identified were D4, D8, D9, B3, G3 and H1. In 2011 in the USA there were 222 cases of measles, 192 (86%) were unvaccinated or had undocumented vaccination status. From 2001 to 2011 in the US 40% of cases were imported, 28% import-linked, 20% import virus associated and 12% of unknown source. The challenges ahead for case classification and diagnosis in countries and regions at or near elimination phase include: outbreaks often include cases of primary and secondary vaccine failure, vaccine reactions are detected and can be confused with disease, collection of appropriate samples for virological surveillance and unknown source cases must be analyzed carefully. To maintain virological surveillance all countries in the region need to continue to strengthen efforts to collect adequate/ appropriate specimens. Additional targets for sequence analysis (expanded sequence windows) are needed to provide greater resolution within genotypes (WHO Lab/Net). Molecular methods will have an expanding role in case confirmation/classification in low incidence settings, additional regional training is needed.

Dr Alberto Severini reported on measles in Canada. In 2011, there were 724 cases of measles in Quebec and 27 cases in the rest of Canada. In 2012, there have been 4 cases. For 2011/2012, 147 samples were genotyped, the genotypes were A (vaccine) (12), D4 (126): 118 were identical to Manchester, plus 8 other sequences, D8 (3), D9 (4) and B3 (2). There were 22 importations in Québec in 2011, 3 of which caused more than 1 secondary case: one case in February exposed in France led to 13 secondary cases (genotype D4), another case in September (no travel history, but genotype B3) led to 4 secondary cases, the index case for the large outbreak led to a total of 686 confirmed measles cases (genotype D4). One hundred and seventeen isolates from the Quebec cases were sequenced (N gene target) and they were all identical D4 sequences (Manchester strain). The Quebec outbreak offers a great opportunity to determine the variability of sequences over time and possibly identifying more sensitive targets for sequencing. The H gene was sequenced from 54 isolates from the Quebec outbreak; all but 2 sequences were identical. A pilot project for real-time measles and rubella surveillance in Canada (MARS Pilot Project) was set up to support real-time, automated Federal/Provincial stakeholder alerting when an investigation is initiated by either the lab/public health and provide centralized Federal/Provincial access to all relevant case investigation data. This would allow
more complete integration of non-nominal lab and epidemiological data. The initial 1-year pilot period was from June 1, 2011 to May 31, 2012. Analysis of the preliminary data is now underway.

**Dr Richard Meyers** presented the global distribution of B3 and D4 genotypes. There are at present in MeaNS 4306 sequences of D4 with 488 distinct sequences and 1598 sequences of B3 with 297 distinct sequences. D4 is still a global problem, there are two lineages related to Enfield and Sistan strains. Looking at selected countries, the Sistan lineage predominates in Russia and Enfield is distributed throughout Europe. The strain naming is based on greater than 50 identical isolates. There was a large recent cluster of B3, there are on-going sequences possibly related to the Football World Cup plus lots of extinct clusters. Analysing the spread of the B3 outbreak that showed significant global distribution a sub-section of the tree had 327 sequences from 129 geographical locations from 2005 to 2012. Summing up the data, D4 continues to dominate MeaNS in terms of sequence number, there are two lineages. Maintenance of Enfield like strain is a function of the massive expansion of this strain. The Sistan strain appears more similar to other expanding strains. Genotype B3 has caused repeated outbreaks; it is difficult to assess the origins of the current UK strain. The strain circulating in southern Africa prior to the World Cup was disseminated widely but that increased following the world cup. Strain naming will be implemented within MeaNS.

**Dr Katsuhiro Komase** presented the data for Japan. The number of measles cases has been decreasing; in 2008 there were 11,015 cases, in 2011 there were 434 cases and to date in 2012 there has been 113 cases. There has been supplementary immunization for the past 5 years targeting teenagers since 2008. Genotype D5 was the predominant genotype from 2006 to 2008. D9, D4, D8 and G3 strains were detected in 2011 and D8, D4, D9, and H1 strains were detected in 2012. Most of them were considered to be imported cases or imported related cases, according to genetic information as well as epidemiologically information in some cases. Two outbreaks caused by D8 strain occurred. One outbreak expanded to more than 20 patients, the index cases were not identified in either outbreak and the sequences were identical. An outbreak caused by D9 strain expanded to 5 patients. Another outbreak caused by H1 strain is now under investigation.

**Session 7: Molecular surveillance continued.**

**Dr Joe Icenogle** discussed rubella virus phylogeny and nomenclature. Currently nomenclature of wild-type rubella viruses contains both mathematically justified and reasonable positions. Continued surveillance is necessary for documenting elimination of genotypes. Current analysis will help to define the process for addressing revisions in nomenclature for wild-type rubella viruses. Genetic characterization has identified 2 clades which differ by 8-10% at the nucleotide level. Clade 1 is divided into 10 genotypes (1a, 1B, 1C, 1D, 1E, 1F, 1G, 1h, 1i, and 1j), of which 6 are recognized and 4 are provisional (designated by lower case letters). Clade 2 contains 3 genotypes (2A, 2B, and 2C). Current analysis is for limited dataset (800+ sequences) and limited sequence window (739 nts). Nevertheless, the process should apply to larger datasets and larger windows. The predominant genotypes currently being identified are 1E, 1G, 1h, 1j and 2B which form 4 phylogenetically distinct groups 1E, 1G/1h, 1j and 2B (boot strap values > 70%). There are phylogenetically distinct groups by this criterion within at least one genotype (2B). Current analysis is for limited dataset (800+ sequences) and limited sequence window (739 nts). Nevertheless, the process should apply to larger datasets and larger windows. Many countries and groups of countries have a non-random distribution of genotypes and some countries have a single predominant genotype. There is statistically significant geographic localization of groups of viruses within phylogenetically distinct groups (e.g. 1h, 1G East Africa and 1G West Africa). Many of these will likely be useful for molecular epidemiology.
Dr Judith Huebschen reviewed sequence variability. The 450 nucleotide hypervariable region (HVR) of the N gene is used for routine genotype determination; sometimes the entire coding region of the H protein is amplified and sequenced in addition. A recent study (Kessler et al., 2011, JCM) also investigated the entire coding region of the P gene to increase the sensitivity of molecular surveillance. Analysis was performed on several genotypes and it was found that D6 strains clustered in both the P and H gene according to their origin (geographical clusters). All but one D4 viruses present in Greece in 2006 belonged to the same transmission chain. D4-Hamburg strains showed some clustering according to the location, but many sequences remained identical confirming a bigger European transmission chain. D4-Manchester showed very little clustering according to country, but rather indicated regular virus exchanges between neighbouring countries. For the D6 viruses the P gene contributed more variation than the H gene, while for the D4 viruses the contribution was mostly similar. Origin of virus (same or different transmission chain) seems to have a far higher impact than evolution during outbreaks lasting for only few weeks/months. For non-identical strains the P and H data mostly confirms separation and taken together provides a more distinct clustering. P and H gene sequencing is certainly useful in some specific situations in which a more refined picture is needed.

**Round table: New generation sequencing**

The main points were:

1. New platforms to be explored
2. Potential to generate lots of genome sequences
3. Need to store information
4. Whole genome sequencing, keep others in loop
5. Keep informed on SharePoint site
6. Developing libraries can be tricky
7. Complementary sequencing between labs
8. Make sure of good understanding of epi circumstances
9. Good strategy needs to be in place

An update in the sharing of sequence data was given by Mr David Featherstone. The WHO database is accessible to everyone, it is password protected and a confidentiality agreement has to be signed before the user can access the data. The database for both measles and rubella is an Excel format. It is based on WHO region/Country/Year of onset/ WHO name/Measles genotype/Epi links/Submitted by/When submitted/GenBank Accession number. Laboratory accreditation includes a timeline for sharing data. Results of virus detection and genotyping are to be completed within 2 months of receipt of specimens and data reported to WHO monthly, for at least 80% of samples appropriate for genetic analysis. Up until 19th June there were 13,312 measles entries and 1,302 rubella entries. There has been significant progress since 2011 with 2,912 new measles virus submitted from 79 countries. Viruses can be submitted either directly to WHO, via MeaNS, through MeaNS via GenBank or direct submission (through SharePoint). There has been 338 new rubella viruses submitted since September.
2011 from 20 countries. WHO SharePoint database currently fulfills a role as a repository for measles and rubella genotypes and allows access to latest versions of procedures and protocols plus links to summary maps, tables and lab addresses.

**Dr Richard Meyers** discussed the MeaNS database and the development of RubeNS database. There is no RubeNS online database as of now but work is in progress, seed datasets have been provided by CDC and Luxembourg, data capture fields are being designed, genotyping is being explored by CDC and analytical tools are being discussed. The rubella database will be similar to MeaNS, however MeaNS works well but is a difficult system to maintain. A better strategy is to build a system from the ground up, using lessons learnt from MeaNS and produce a system for Rubella that could be modified for measles. Much of the new system will be developed to be generic. The MeaNS data base has 9,798 samples, 9,632 N_450 sequences and 592 H sequences. There are a total of 183 users, with 53 people who submit data. The peak year for submitting data was 2011. New features include a training video, WHO map and document sharing. MeaNS continues to function well, some bugs in the system have been removed and it has become a valuable data resource.

**Polio eradication initiative: Programmatic use of molecular surveillance.**

**Dr Mark Pallansch** discussed the polio program. There is no global database of polio sequences. Molecular epidemiology takes into account: the virus isolate, genomic sequence data, analysis, interpretation, inference and informatics. Identical sequences between isolates from different patients are extremely unusual. If there is an epidemiological linkage, same district, same month; then identical VP1 is possible. Identical sequences from epidemiologically unlinked sources are usually a contaminant. Progress towards elimination is shown by reduction in number of cases, reduction in the geographic extent and reduction in genetic diversity. Sequence information tells you more than just where the wild virus is found, the quality of sequence information is dependent on the quality of all aspects of surveillance. Not all interpretation of sequence data is obvious.

**Dr Cara Burns discussed the use of polio molecular data: Timeliness, Reporting, Mapping and Limitations.** The WHO GPLN standard requires complete VP1 capsid protein sequencing of all wild polioviruses and VDPVs from polio cases. The sequence QA/QC system ensures high-quality sequence data. Poliovirus classification is based on the VP1 capsid region. Serotype (>25% nucleotide divergence), Genotype (>15% nucleotide divergence), Genetic Cluster (>5% nucleotide divergence); cluster designations are evaluated in June each year. Poliovirus has one of the highest virus evolution rates (~1% per year) which allows the poliovirus to be tracked and permits the estimation of the timing of virus circulation events. Sequence results are reported to WHO and partners. Diagnostic reports contain the virus serotype and classification (Wild Poliovirus, Vaccine-derived poliovirus (VDPV), Sabin poliovirus). Analysis of genetic cluster and closest sequence match is performed. Monthly reports are based on phylogenetic analysis. Complete VP1 sequence is required for the PEI. Phylogenetic analysis used to distinguish “source reservoirs” from non-reservoir “indicator communities” Sequence data are used to drive eradication Program (target reservoirs) and identify surveillance gaps. In summary the rapid evolution of poliovirus nucleotides allows the virus to be tracked using high-quality VP1 capsid sequence data. Sequencing information is integrated into the PEI. Analysis and comparison of poliovirus capsid sequences combined with epi data allows the elucidation of events such as genetic diversity estimates, importations within a country, between countries and between continents, re-established transmission within a country, identification of surveillance gaps (orphan viruses) and identification of separate emergence events that result in independent VDPV lineages.
Dr Steve Oberste reported on Polio QC/accreditation of molecular tests. Expansion of rRT-PCR capacity has progressed with Polio real-time PCR currently used in >50 labs worldwide. Regional training workshops are ongoing, with training of 20 China provincial CDC polio labs in 2012 and additional workshops planned in 2012-2013. Standardized methods have recently been introduced with basic kits and supplemental kits provided. QA for molecular testing is performed. Kits for NAPH, RT-PCR, rRT-PCR, and sequencing are distributed by CDC. This contains primers, probes, buffers, etc., everything except enzymes. Standard protocols have been written. Accreditation checklists for virus isolation, ITD (all methods included), and sequencing. Annual proficiency testing is performed. The panels test the proficiency of ITD laboratories in polio NAPH, conventional PCR, rRT-PCR, sequencing. There are 10 samples per panel and separate panels for NAPH, PCR, and sequencing. PCR accreditation is based on proficiency testing and assesses proficiency in detecting wild, Sabin, and VDPV strains. Labs are required to send data files to insure correct interpretation. There is feedback to the laboratory to help with troubleshooting if there is an issue. There is also an on-site review. Test interpretation is taken into account along with reporting and timeliness. Sequencing accreditation by proficiency testing was introduced in 2011 and scoring “counts” in 2012. This program assesses proficiency in PCR methods, sequencing reactions, fragment assembly, sequence editing and interpretation.

Dr Mark Pallansch discussed the proposal to develop a Polio nucleotide sequence database. Current ability of the GPLN to collectively respond with timely sequence information and analysis to meet program needs has been demonstrated repeatedly. However, polioviruses isolated and sequenced outside the GPLN do not always have rapid or appropriate analysis and reporting. Currently a repository does not exist for poliovirus sequences for use by GPLN or other interested parties. A growing level of concern by some GPEI partners that lack of access to poliovirus sequences may represent an impediment to polio eradication. The GPLN functions as a coalition of multiple independent agencies and individuals, acting through consensus under overall WHO coordination with support from multiple sources. There is a need to provide appropriate record of data and analysis of polio sequences from significant epidemiologic and virological episodes, continue and accelerate efforts to generate scientific publications and where possible, deposit annotated poliovirus sequences in public databases with the consent of national programs and laboratories involved with generating the data. There is a need to create a system to collectively share poliovirus sequences to enhance GPEI objectives. Rather than create a novel system for polio sequence data, the example of MeaNS can be used to develop an analogous system and take advantage of similar needs for rubella sequences (RubeNS).

Discussion of Polio surveillance

The main points were:

1. Criteria for eliminating genotypes. Viruses are kept on the tree, listed as presumed gone. It may come back.
2. Record last case of genotype and continue surveillance
3. Assessment of RT-PCR only assessed on PCR not whole protocol.
4. 3 year window time with comprehensive tree.
5. Environnemental surveillance is a supplement to AFP surveillance
6. Supplemental surveillance activity may be successful in detecting viruses that have been missed by AFP surveillance i.e. Orphan viruses.

Session 8: Update on Mumps

Dr Li Jin discussed the new nomenclature of the mumps virus and the sequence database. To update the data a collection of >1250 sequences including 65 genomes, 120 HN and 500 SH was utilised. Sequence clustering and phylogenetic analysis was performed using a range of methods, with two reference strains for each genotype were selected. Modifications were made to the previous nomenclature. HPA has instigated a mumps strain bank. MuV isolates including majority of the reference strains are available. HPA has generated a local MuV sequence database, which will facilitate tracking of sequence variants of MuV and their transmission. Submission of sequence information to the Database & GenBank is highly recommended. Global genotype and nucleotide databases similar to those for measles and rubella at WHO/HQ will be considered. Mumps diagnosis: current mumps diagnostic tests present a challenge to identify mumps cases, especially in previously vaccinated individuals. Because of the lower positive predictive value of IgM testing in cases with a history of vaccination or previous infection, laboratories with capacity for RT-PCR should consider including this diagnostic method in addition to IgM testing. Countries may consider using oral fluid sample collection to enhance the sensitivity of laboratory confirmation of mumps as the same sample can be used for both IgM detection and RT-PCR. WHO LabNet can provide protocols upon request. In summary: A new nomenclature of mumps virus is published, WER 87(22): 217-24, 2012. Recommendations have been made to encourage mumps surveillance. Guidelines have been proposed for genotyping mumps viruses and reference sequences are available as a base for routine genotyping. The WHO-Mumps working group/LabNet will provide protocols upon request.

The challenge of mumps diagnosis was presented by Dr Paul Rota. RT-PCR and cell culture are the best diagnostic tests currently available. Serological tests for mumps IgM are more widely available but less sensitive. Persons with a history of mumps vaccination may not have detectable mumps IgM antibody regardless of the timing of specimen collection. The ability to detect IgM varies by vaccination status and is highest in unvaccinated persons (80%–100%), intermediate in one-dose vaccine recipients (50%–80%), and lowest in two-dose vaccine recipients (14%–50%). Absence of a mumps IgM response in a vaccinated or previously infected individual presenting with clinically compatible mumps does not rule out mumps as a diagnosis. A RT-PCR has been developed for the N gene. The reagents and methods are available from CDC. The majority of cases are genotype G. This assay has a lower limit of detection of approximately 10 copies of mumps N gene RNA per sample. The specificity of this assay N gene rRT-PCR assay was evaluated by testing samples from sporadic cases of parotitis. Laboratory testing should be used to confirm suspected outbreaks of mumps, but not to confirm every suspected case. Laboratory testing should only be conducted on suspected cases meeting a stringent case definition. Because of the limited utility of serologic testing, laboratories with capacity for PCR should consider establishing the mumps real time RT-PCR assay as a diagnostic method. Samples should be collected to establish a genetic baseline for wild-type mumps.

Dr Albert Severini discussed Mumps genotypes in Canada in 2011/12. Mumps has been nationally reportable since 1924, reporting mandated by provincial and territorial (P/T) legislation. Only confirmed cases are nationally reported. Passive reporting is through the Canadian Notifiable Disease Surveillance System (CNDSS). Routine infant MMR immunization was introduced by 1983. A routine 2nd dose of MMR vaccine in 18 months or school entry was introduced by 1996. Catch-up
campaigns have been held in most provinces. For 2011/12 a total of 147 samples were genotyped. The genotypes were D (1), F (4), G (136, 123 identical), H (5) and N (1). In summary every year, genotype G (Sheffield) is predominant. Canada has the capacity to collect good molecular data for mumps outbreaks but the molecular/epi links are still not as strong as those for measles and rubella surveillance.

Dr Jean-Marie Okwo Bele, Director of the WHO department of Immunization, Vaccines and Biologicals spoke in the closing ceremony. He thanked David Featherstone for his efforts in building up the laboratory network to the high standard that it is today. The first LabNet meeting had only one region (AMR), there are now 6 regions fully functional, this could not be achieved without dedicated effort and passion. The list of activities includes: development of laboratory manuals, training workshops, reporting procedures, regional LabNet meetings, accreditation program, and validation of new techniques and collection of data. This covers lots of detailed work done well with lots of patience. There are not enough words to sincerely thank David.

Recommendations Final (16 August 2012)

1. The use of oral fluid in the 5 AFRO trial countries has been successfully evaluated and the decision on implementation of using OF samples to enhance molecular surveillance is awaited. LabNet should be prepared to develop further training materials and consider electronic media such as video to assist in training surveillance staff. **Action: CDC, WHO, HQ. Timeline: Mid-2013**

2. The Global Measles and Rubella Laboratory Network Accreditation Checklist will undergo review with the revised checklist to be implemented, starting from January 2013. **Action: WHO HQ and RCs Timeline: September 2012**

3. The Global IgM PT results for 2011 showed the high quality of the testing in LabNet but identified some issues with individual laboratory's sensitivity in a small number of situations. Laboratory coordinators and RRLs should investigate the laboratories which report variant results to address possible laboratory or assay issues. **Action: WHO RCs, HQ, RRLs. Timeline: On-going**

4. One of the hallmarks of LabNet is the strong commitment to quality control. Current quality control and laboratory accreditation protocols focus primarily on serologic testing. Considering the expansion of molecular testing capacity within LabNet, it is now necessary to initiate a proficiency testing program (molecular PT) for molecular testing. A framework for developing a molecular PT program was presented during the meeting and an electronic copy is being distributed with the meeting materials. The key questions for developing the molecular PT panel are highlighted on the document. LabNet members should read the document and include comments and suggestions to address the key questions. Responses should be sent to WHO/HQ with copy to CDC not later than 1
August, 2012. **Action:** WHO RCs, HQ, LabNet. **Timeline:** revised protocol to be returned to WHO RCs, RRLs, and GSLs by mid-September. Final protocol approved by the end of 2012

5. Many regions are developing criteria for verification of elimination of measles, rubella and CRS. Several of the criteria address the completeness of surveillance and laboratory performance. It is critical that LabNet laboratories be involved in the development of these verification criteria. In addition, the criteria that are used to evaluate laboratory performance and surveillance should be harmonised as far as possible between regions. Regions are encouraged to share their draft documents with the LabNet coordinators and LabNet partners for comment before finalisation. **Action:** WHO Regional Offices, Lab Coordinators. **Timeline:** On-going

6. Analysis of the sequences of the measles H and P genes can help to differentiate viral lineages with the same N-450 sequences. LabNet laboratories are encouraged to consider H and P sequencing when additional resolution of transmission pathways is required. LabNet laboratories should be able to access the protocols for sequencing the H and P genes on the SharePoint website. In addition, next generation sequencing technologies offer the possibility of rapidly increasing the amount of sequence data available for measles and rubella viruses. Some LabNet laboratories will be testing these methods in the coming year. LabNet members are encouraged to share their experiences and protocols by posting updates on the WHO SharePoint site. **Action:** GSLs, RRLs, WHO/HQ LabNet. **Timeline:** On-going

7. Viral lysates and clinical samples for measles and rubella can be stabilized by drying onto FTA cards. These samples are non-infectious and can be shipped at ambient temperature. Countries are encouraged to use FTA cards for shipping these samples when other options are not practical or financially non-viable. Protocols will be posted on the WHO SharePoint site. **Action:** WHO/HQ LabNet. **Timeline:** On-going

8. CDC has developed a well characterised and sensitive real-time RT PCR protocol for rubella virus detection which detects all rubella virus genotypes. LabNet is encouraged to use this protocol or a similarly well characterized and sensitive method for rubella virus identification. **Action:** WHO/HQ LabNet. **Timeline:** On-going

9. Laboratories are reminded that the standard 739 nt sequencing window in the E1 coding region is the minimum which should be used for molecular epidemiology of rubella viruses. The proposed RubeNS database will accept sequences which are shorter than this but they will not be used for further analysis. **Action:** WHO/HQ LabNet. **Timeline:** On-going
10. Countries lacking recent baseline genetic data for endemic measles and/or rubella viruses, should be assisted with developing a plan of action for obtaining this data, with prioritisation for countries planning to initiate increased rubella control strategies in the next 4 years and those countries which have yet to establish regular M&R sequence data collection and reporting. Regional capacity to generate sequence information should be strengthened, especially in AFR. Referral of appropriate specimens to the designated RRL or GSL should be considered for those countries without the capacity to perform sequencing. **Action:** Regional Office Lab Coordinators, WHO/HQ, RRLs, LabNet. **Timeline:** LCs to develop PoAs by end 2012, then on-going

11. In order to provide support to National Labs and countries for collecting samples suitable for molecular analysis, Regional Lab coordinators are encouraged to inform RRLs responsible for supporting countries which are experiencing outbreaks. **Action:** Regional LCs, RRLs, **Timeline:** On-going

12. The nomenclature for wild-type rubella viruses should be updated and reported in the WER and include an outline of the process for future updates. The updates should focus on currently circulating viruses and definitions of phylogenetically distinct and geographically localized groups of viruses. Data from the proposed RubeNS database should be used as the basis for future nomenclature updates. **Action:** CDC, WHO/HQ, RubeNS working group, LabNet. **Timeline:** WER published by end 2012

13. The recent outbreak of rubella in Tunisia, which resulted in a reported rate of meningoencephalitis much higher than expected, should be fully investigated and a summary presented at the next LabNet global meeting. **Action:** Tunis RRL, EMR RLC **Timeline:** Reported next meeting

14. Current mumps diagnostic tests present a challenge to identify mumps cases, especially in previously vaccinated individuals. Because of the lower positive predictive value of IgM testing in these cases, laboratories with capacity for RT-PCR should consider including this diagnostic method in combination with IgM testing. Countries may consider using oral fluid sample collection to enhance the sensitivity of laboratory confirmation of mumps as the same sample can be used for both IgM detection and RT-PCR. **Action:** LabNet. **Timeline:** On-going

15. A sequence database similar to MeaNS and the proposed RubeNS will be considered if resources allow. In the interim, all mumps virus sequences, including multiple sequences detected in same location and time, should be submitted to GenBank and labs encouraged to also inform HPA of their submissions. WHO will develop a Mumps SharePoint database similar to that for measles and rubella. **Action:** WHO/HQ, LabNet. **Timeline:** On-going
16. An ad hoc mumps steering group is being formed to advise the WHO LabNet on decisions relating to mumps nomenclature and diagnostic testing methods. Decisions relating to changes in the nomenclature of MuV or the nomination of new genotypes will be shared with the broader group of laboratories with an on-going interest in mumps to generate a consensus prior to submission for publication. LabNet members should be aware of the recent changes to the mumps nomenclature that were published in the WER. The updated reference strains and genotype assignments should be used immediately. **Action:** LabNet. **Timeline:** On-going

17. LabNet should consider validating alternative serologic methods for measurement of population immunity to measles and rubella. In particular the Luminex platform offers a reliable means to measure immunity to multiple antigens in a high throughput format. **Action:** GSLs, RRLs, WHO/HQ. **Timeline:** On-going

18. Consideration should be made for the 2013 Global LabNet meeting to be held in one of the WHO regions, with subsequent ones being held on a rotating basis in the other regions. **Action:** WHO/HQ, RLCs LabNet. **Timeline:** Mid June 2013.