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for the Measles/Rubella
National Reference
Laboratories of Western and
Central European Countries

**5-7 May 2014
Helsinki, Finland**

MEETING REPORT

**WHO Subregional meeting for the
measles/rubella national reference laboratories
of western and central European Countries**

Helsinki, Finland, 5-7 May 2014

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Executive summary

WHO Regional Office for Europe convened a technical meeting at the National Institute for Health and Welfare (THL) with the support of the Finnish Ministry of Social Affairs and Health in Helsinki, Finland on 5-7 May 2014. The meeting aimed at reviewing the status and performance of the European Measles Rubella Labnet, particularly in the context of approaching measles and rubella elimination, with the following objectives:

- present updates and exchange information on the recent progress of the European Regional Measles-Rubella Elimination goal both from WHO and country perspective, primarily focusing on accreditation issues and laboratory contribution to annual country reports to the Measles Rubella Regional Verification Committee;
- provide a training opportunity on MeaNS and RubeNS databases, in order to support the intensification of measles and rubella molecular epidemiology surveillance;
- discuss challenges and strengthen the commitment of Measles Rubella Reference Laboratories to the WHO European regional goal for the Elimination of Measles and Rubella

The meeting brought together representatives and observers from 37 countries of the WHO European Region and the United Nations Administered Province of Kosovo (in accordance with Security Council resolution 1244 (1999)), from the Regional Reference Laboratories (RRLs) in Germany and Luxembourg, the Global Specialized Laboratory (GSL) in the United Kingdom, the European Centre for Disease Prevention and Control (ECDC) and from WHO headquarters and the Regional Office for Europe.

The six sessions allowed covering several topics: global, regional and national updates, accreditation and laboratory performances issues, role of laboratories in verifying measles/rubella elimination and molecular epidemiology/surveillance.

Globally the number of measles cases has been reduced significantly during the recent 12 years, although vaccine coverage has levelled at 84%. All 53 countries of the WHO Region for Europe use MMR vaccines in a two-dose schedule. Insufficiently immunized individuals can give rise to outbreaks. At global level, elimination of rubella is lagging behind measles elimination. A global network of 711 laboratories conducts surveillance of measles and rubella. At the European level, 72 laboratories are participating in this network. All countries of the EU/EEA report measles and rubella data to ECDC through TESSy, from where the data is shared with WHO.

Guidance on the selection of specimens and the use of laboratory methods was presented, and much attention was given to the interpretation of laboratory results. In 2013, European laboratories have performed very well in proficiency testing.

The importance of obtaining sequence information from circulating viruses was underlined, and participants received instruction as to how to use the genetic information databases available. Laboratories were also encouraged to send samples for isolation or attempt isolation of viruses and to submit isolates to the reference laboratories and GSLs.

National Committees for the Verification of Measles and Rubella Elimination have been established in 41 countries and the majority of those have submitted reports to the Regional Verification Commission (RVC). Based on 2010-2012 reports, the RVC concluded that endemic transmission of measles has been interrupted in 15 countries, and rubella no longer circulates in 18 countries.

Participating countries presented their respective surveillance data and information about how their laboratories perform when cases of measles or rubella are identified. An increasing number of laboratories produce and share molecular data. Decreasing government funds is identified as a major challenge in many laboratories. Much effort is taken in enhancing collaboration between the laboratory, epidemiologists, and public health officers. It has been recognized that health care workers need to be encouraged to get properly immunized in order to prevent transmission of measles and rubella in health care settings. Surveillance of the congenital rubella syndrome needs to be intensified.

Diagnosis of measles and rubella in vaccinated patients often presents a challenge, and additional testing methodologies should be implemented in the diagnostic work-up of such cases.

Efforts need to be taken towards improving timeliness and completeness of data reporting to WHO. To this end, the Measles Rubella Laboratory Data Management System is being further developed. With decreasing numbers of measles and rubella cases, laboratories need to remain vigilant, maintain a high level of proficiency but they also need sufficient support in order to be able to fulfil their duties.

Progress, challenges and lessons learned were reviewed throughout the meeting, and solutions and future plans were discussed. The key recommendations from the meeting are summarized below:

- 1. Mobilize countries' and partners' political commitment and accountability for maintaining a proficient and sustainable Measles and Rubella Labnet**
- 2. Improve measles /rubella laboratory and epidemiological data integration and strengthen case-based reporting as a key contribution to verifying measles and rubella elimination**
- 3. Comply with WHO MR Labnet standards and maintain sufficient performances enabling full accreditation and contribution to the verification of MR elimination**
- 4. Enhance molecular surveillance of measles and rubella viral sequences and scale up the reporting to MeaNS and RubeNS databases**

29 of the 50 participants completed a questionnaire evaluating the meeting. Their feedback on the usefulness of the sessions, the organization and their overall impressions about the meeting were overwhelmingly positive.

1. INTRODUCTION

WHO/Europe coordinates the European Measles and Rubella Laboratory Network (MR Labnet) to facilitate high-quality laboratory investigations of measles and rubella in the region and to support Member States' efforts towards measles and rubella elimination.

The European MR Labnet consists of WHO-recognized laboratories located at different levels: one global specialized laboratory (Public Health England, United Kingdom), three regional reference laboratories – Robert Koch Institute (Germany), Centre de Recherche Public de la santé (Luxembourg) and Gabrichevsky Institute of Epidemiology and Microbiology (Russian Federation)- and 68 national and subnational laboratories in most Member States.

WHO Regional Office for Europe convened a technical meeting in Helsinki, Finland on 5-7 May 2014 to review the status and performance of the European MR Labnet. The scope of the meeting was to:

- present updates and exchange information on the recent progress of the European Regional Measles-Rubella Elimination goal both from WHO and country perspective, primarily focusing on accreditation issues and laboratory contribution to annual country reports to the Measles Rubella Regional Verification Committee;
- provide a training opportunity on MeaNS and RubeNS databases, in order to support the intensification of measles and rubella molecular epidemiology surveillance;
- discuss challenges and strengthen the commitment of Measles Rubella Reference Laboratories to the WHO European regional goal for the Elimination of Measles and Rubella

The meeting was attended by:

- Representatives of the Measles/Rubella National Reference Laboratories from the following countries: Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Cyprus, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, the former Yugoslav Republic of Macedonia, Turkey, and the United Kingdom (the invited representative from Croatia was unable to attend the meeting);

- Representatives of the WHO Measles/Rubella Regional Reference Laboratories in Germany and in Luxembourg;
- A representative of the WHO Measles/Rubella Global Specialised Laboratory in the United Kingdom;
- A representative of the European Centre for Disease Prevention and Control (ECDC)
- Observers from Kosovo (in accordance with United Nations Security Council Resolution 1244 [1999]) and the European Centre for Diseases Prevention and Control (ECDC) in Sweden;
- Representatives from WHO headquarters and from the WHO Regional Office for Europe;

This report summarizes the presentations given by laboratory representatives and technical experts and provides the recommendations drawn from the exchanges and discussions that took place during the meeting.

2. SESSIONS OF THE MEETING

The Finnish Ministry of Social Affairs and Health (MoH) hosted the meeting held at the National Institute for Health and Welfare (THL) in Helsinki, Finland. In his welcome address, Mikko Paunio from the ministry reminded participants that measles control and elimination turned out to be much more difficult than anticipated in the 1960s when this goal was first envisioned. He also mentioned that Finland was the first country that introduced a two-dose vaccination policy for measles, rubella, and mumps in the early 1980s. In addition, he pointed out that techniques for the measurement of antibody avidity, a central method used in the laboratory surveillance of measles and rubella was originally developed in Finland.

Mika Salminen briefly wished meeting participants welcome on behalf of THL.

Myriam Ben Mamou (Measles/Rubella Laboratory Coordinator and meeting chair, WHO/Europe) presented the objectives and the expected results of the meeting.

SESSION 1: GLOBAL AND REGIONAL UPDATES

Chair: Annette Mankertz (WHO RRL, Berlin)

1.1 WHO updates

1.1.1 WHO Global update – Mick Mulders (WHO headquarters)

While global measles vaccine coverage has levelled off at 84%, between the years 2000 and 2012 a reduction of measles incidence by 77% could be accomplished. The current measles incidence is 33 cases per one million persons and the foreseen target is to reduce this incidence to less than 5 cases per one million persons. Rubella-elimination appears to lag behind measles elimination, particularly in the AFRO and

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SEARO Regions. Also, much less information on rubella is available compared with data available for measles.

In 2013, 151 WHO Member States have reported measles cases and 111 Member States have reported laboratory-confirmed measles cases. Laboratory data is reported by 149 and genetic data by 45 Member States, respectively. Global elimination targets set for 2015 are unlikely to be met, but the Global Vaccine Action Plan strives for the elimination of measles and rubella in five WHO regions by 2020. At present 711 subnational, national, and regional laboratories form a strong network that contributes to measles and rubella surveillance. These laboratories have reported 73,163 laboratory-confirmed measles cases in 2013. The network laboratories have tested 145,000 serum specimens for the presence of measles-specific IgM antibodies, of which 46,000 were classified as positive. Corresponding numbers for rubella are almost 98,000 sera tested and approximately 10,000 rubella IgM-positive findings. Working towards elimination of these two viruses, it is important to get, besides serological results, also data on the molecular characteristics of these viruses.

Key challenges in measles and rubella elimination are to achieve interruption of endemic transmission of the two viruses. The risk of importation persists. In many countries, there are growing numbers of adults who have escaped natural infection but have not been covered by vaccination programs, or who object to vaccination. These susceptible individuals, together with insufficiently immunized persons, can give rise to outbreaks. Many countries have competing health priorities, which may postpone or delay measles and rubella elimination plans. Particularly in the EMRO Region, security issues are presenting major obstacles to the successful completion of vaccination programs.

1.1.2 Measles and rubella situation and surveillance in the WHO European Region – Mark Muscat (WHO/Europe)

Countries of the WHO European Region reported 31,685 measles cases, including 7 cases with fatal outcome, in 2013. One third of these cases were observed in individuals ≥ 20 years of age. Most cases with known vaccination history were unvaccinated, but for a large fraction of cases, information about vaccination status was not available. Outbreaks have been observed in health care settings and in schools. Also in the European Region certain groups of the population are not vaccinated because they are difficult to reach or they refuse vaccination. Almost 40,000 rubella cases have been reported during the same period.

All 53 countries of the Region use measles-mumps-rubella (MMR) vaccines in a two-dose schedule. However, intervals between the first and the second dose vary between one month and 12 years.

Towards the elimination goal, to date, 41 countries have established a National Verification Committee, while in 12 countries this process is pending. Thirty-three countries submit annual status reports. Thus far, 15 countries have interrupted measles transmission and 18 countries rubella transmission. Progress has thus been made but

the WHO European Region is off track towards the elimination goal set for the year 2015.

1.1.3 WHO European Measles and Rubella Laboratory Network – Myriam Ben Mamou (WHO/Europe)

The European LabNet currently consists of 72 laboratories of which 68 national and subnational laboratories. 64 have been fully and three provisionally accredited, respectively. For one laboratory, accreditation is pending.

During 2013, LabNet laboratories received almost 60,000 specimens, and serum was the most common specimen type. The rate of laboratory investigation of measles cases varied among countries and only a limited number of measles sequences have been reported from several high incidence countries in 2013. Poland reported most rubella cases, but only a minor fraction of those have been laboratory-confirmed.

Timeliness and completeness of reporting, as well as accreditation issues for some laboratories, remain a problem. As we are progressing towards measles and rubella elimination, challenges also include the need for more sequence data and financial sustainability of Labnet.

1.2 Reference laboratories updates

1.2.1 Global specialized laboratory update (London)– Kevin Brown (WHO Measles/Rubella Global Specialised Laboratory, London)

In addition to its global function, the laboratory also serves as a National Reference Laboratory for measles and rubella within Public Health England (PHE) for the United Kingdom, and as a Regional Reference Laboratory for Ireland. The laboratory undertakes confirmatory testing of samples submitted by other laboratories, and attempts to genotype all samples from the United Kingdom. By PCR, measles virus is detectable in oral fluids (OF) a few days before onset of rash, during the entire period of rash, and even a few days thereafter. OF is thus the optimal sample for virus detection but also for the detection of virus-specific IgM and IgG antibodies. Plans to contract measles and rubella PCR diagnosis to regional laboratories within the United Kingdom may reduce the number of specimens received by the GSL.

The GSL collaborates in the development of a molecular external quality assurance program for measles and rubella. To this goal, a validation panel has been produced and tested with good overall results. A triplex PCR assay for measles including primers for the H and N genes and B2M as control has been successfully evaluated. PHE hosts the measles (MeaNS) and rubella (RubeNS) databases for WHO. Genotyping of measles virus is performed from a 450 nucleotide fragment at the C-terminal end of the N gene. Ten full genome sequences of measles virus have been completed by whole-genome sequencing so far. Benefits from whole-genome sequencing will be assessed, in order to determine the mutation rate in circulating measles viruses and to detect trends in mutation events.

The GSL maintains a virus strain bank which currently includes 155 measles and 27 rubella virus isolates, respectively.

Some problems with commercial assays for the detection of rubella antibodies have been noted. Some of these problems are likely due to the instability of rubella antigens used in these tests.

The GSL uses neutralization test for seroepidemiological studies, but this assay is time-consuming and expensive to perform. ELISA tests are used as alternative. The GSL has successfully competed for funds from the Gates Foundation to be used towards the development of a point-of-care test for tetanus and measles IgG antibodies. Promising results have been obtained. Such a test could be used in surveillance.

1.2.2 Regional Reference Laboratory update (Berlin) – Annette Mankertz (WHO Measles/Rubella European Regional Reference Laboratory)

Re-testing at RRL Berlin of samples sent from National Laboratories produces results that are in agreement with those obtained locally in almost all instances. This finding shows the high quality work of National Laboratories. The proficiency testing conducted in 2013 further underlines the expert performance of National Laboratories, as all of them passed successfully for rubella and only one laboratory failed for measles. Serum samples for retesting by the RRL Berlin should be shipped in vials provided by the RRL. Increased sampling for virus genotyping is encouraged. Samples for genotyping should be collected on FTA Classic Cards in a letter to avoid high costs of shipping on dry ice and biosafety issues. Sufficient patient information must be provided with the sample, including gender, age, vaccination status, date of symptom onset, and date of sample collection. If an outbreak continues, repeat samples should be collected and provided to the RRL at reasonable intervals. Measles genotype D4 Hamburg has been circulating in Europe, and a summary of the epidemic caused by this virus has been published (<http://www.ncbi.nlm.nih.gov/pubmed/21801615>).

1.2.3 Regional Reference Laboratory update (Luxembourg)- Judith Hübschen (WHO Measles/Rubella European Regional Reference Laboratory)

During 2013, 25 proficiency panels and 282 filter papers have been distributed to National Reference Laboratories. All of the 27 participating laboratories had returned excellent results from measles testing; a few laboratories had discrepant results with rubella testing. It has been noted that a commonly used commercial rubella IgM test occasionally yields false-positive results. A number of different test kits are used in the network. Approximately 1,000 samples are submitted to the RRL for retesting; of those approximately 40% are for measles and 60% for rubella testing. The quality of samples in decreasing order: liquid serum (approximately 60%), dried serum spots; dried blood spots; oral fluid (<5%). National Laboratories are encouraged to submit samples for genotyping of measles and rubella viruses. This is partly hampered by the lack of appropriate clinical specimens for rubella genotyping.

Discussions:

- Information about the measles and rubella situation in the Russian Federation was required. Many cases are reported from adults, particularly belonging to certain religious groups. There appear to be many small outbreaks predominantly in three large regions of the Russian Federation.
- The quality of rubella surveillance in the region has been questioned. Several countries do not have a national surveillance program for rubella. Most rubella cases are reported from Poland, but only few of those are laboratory-confirmed. Vaccination programs for boys have been established in Poland in 2003, and thus, a large proportion of males are susceptible to rubella. Rubella surveillance is hampered by the fact that a significant fraction of rubella infections are subclinical or symptoms are very mild.
- Surveillance of congenital rubella syndrome is weak in many countries. No data is available as to how many pregnancies have been terminated because of probable or confirmed rubella infection. Romania has experienced simultaneous outbreaks of measles and rubella.
- Since vaccination will result in an IgM response, recently vaccinated patients with measles and/or rubella positive IgM findings should be classified accordingly in databases. Equally important to mention is that vaccine-derived sequences should not be uploaded to MeaNS or RubeNS databases.
- Some rubella IgM tests yield negative results when samples are collected late or remain in transport for extended periods.
- The point-of-care test developed at the GSL is still under validation and is currently being evaluated in African outbreak investigations.
- Viral nucleic acids suitable for PCR and genotyping are stable on FTA cards for at least two months. Viruses are inactivated and not infective, so shipping of FTA cards is cheap and simple. Protocol for collection specimens on FTA cards and shipment of such specimens should be shared within the network.
- Genotyping should be performed and reported within two months after a positive case has been identified. N-gene sequencing gives all the required information for measles surveillance. Sequencing of other genes does not provide additional essential information for surveillance. At the moment, only global and regional reference laboratories are participating in EQA for molecular testing. A kit for that purpose should be available for other laboratories by autumn 2014..
- Proficiency panels for serological testing usually contain samples yielding very high OD levels by ELISA-based assays. Also sera yielding lower reactivity should be included in such panels. This was regarded as an important suggestion. However, some laboratories have difficulties in correctly identifying low-positive samples. The confirmatory testing program provided by the RRL will help solving these problems.

SESSION 2: VERIFYING MEASLES AND RUBELLA ELIMINATION

Chair: Kevin Brown (WHO GSL, London)

2.1 Update from the Regional Verification Commission for measles and rubella elimination – Irja Davidkin (THL, Helsinki)

As of May 2014, 41 National Committees with chairpersons have been established. Thirty-six reports have been submitted to the Regional Verification Commission, of which 33 have been reviewed. Lines of evidence for elimination are composed of data on population immunity, epidemiological data, information from genotyping, sustainability of immunization programs, as well as of supplementary evidence. Endemic transmission of measles has been interrupted in 15 countries: Armenia, Azerbaijan, Belarus, Bulgaria, Cyprus, Czech Republic, Estonia, Finland, Israel, Kyrgyzstan, Latvia, Luxembourg, Netherlands, Portugal, and Slovakia. Rubella transmission has been interrupted in addition to these 15 countries also in Croatia, Ireland, and in the Republic of Moldova. The absence of endemic measles and rubella cases, the presence of a high-quality surveillance system, and evidence from genotyping are essential criteria supporting the interruption of endemic transmission, as detailed in the 2014 Framework for the verification of measles and rubella elimination (WHO/Europe). Timeliness of notification, and the rate of suspected cases that tested negative for measles and rubella IgM count for alternative indicators. Information about population immunity against measles and rubella, and sustainability of national immunization programs are essential factors in the elimination program. In some status reports quality of surveillance data has been found inadequate. Occasionally there are inconsistencies between epidemiological and laboratory data, and sometimes, essential data is missing from reports.

2.2 Measles and Rubella surveillance and reporting in EU – EEA – Sabrina Bacci (ECDC, Stockholm)

ECDC's primary function is to identify, to assess and to communicate current and emerging threats from communicable diseases to human health. The objectives of the EU Measles-Rubella surveillance coordinated by ECDC are to monitor outbreaks in the EU; to provide timely data to support the elimination of these diseases by 2015; to strengthen surveillance systems in Member States; to raise awareness regarding the resurgence of these diseases in order to encourage Member States to reach and maintain 95% vaccination coverage. Measles and rubella data collected by ECDC is case-based and not specimens-based. ECDC data is compatible with the WHO Surveillance Guidelines for Measles and Rubella updated in December 2012. ECDC transmits data to WHO on a monthly base. All 30 EU/EEA countries report to ECDC through TESSy. The laboratory variables collected through TESSy cover the date of

specimen collection; the date of laboratory result; the genotype of the virus; results from IgM and IgG tests (and for rubella results from IgG avidity test); results from virus detection or isolation; information on the type of specimen for serological analysis or for virus detection. Data stored in TESSy is only visible to the reporting country but not to other countries. ECDC provides feedback to Member States through monthly updates on the ECDC website and through a quarterly “monitoring report”. An increasing proportion of cases reported from EU/EEA Member States are from unvaccinated individuals. During 2013, almost 40,000 cases of rubella have been reported to ECDC, almost all of them from Poland. ECDC plans to revise case definitions and to support Member States in reporting CRS cases.

SESSION 3: SITUATIONAL REPORTS

Chairs: Sabine Santibanez (WHO RRL, Berlin)

Judith Hübschen (WHO RRL, Luxembourg)

Representatives and observer from each participating country and UN administered province of Kosovo reported on their laboratory’s performance in EQA, in timeliness of reporting, and presented details on confirmed cases of measles and rubella during the years 2012, 2013, and the first months of 2014 (See Annex 1). Participants presented the following strengths, challenges, and future plans of their laboratory and national programs:

Strengths

- Well-established laboratory experience with involvement in research projects;
- Most laboratories have achieved accreditation;
- Close cooperation between laboratory and epidemiologists;
- Endemic circulation of measles and rubella has been interrupted in many countries;
- A variety of different laboratory methods available, which allows for the differential diagnosis of suspected measles or rubella cases;
- Many laboratories performed very well in EQA;
- Some laboratories have well-established molecular methods for detection and sequencing;
- National action plan for measles and rubella elimination;
- National Verification Committee established;
- SIAs to increase vaccination coverage;
- Vaccination programs for individuals who have not been immunized during childhood;
- Including mumps in surveillance activities;

Challenges

- Strengthen national surveillance systems for measles and rubella;

- Decreasing government funds and limited access to external funding;
- Insufficient samples received from suspected measles or rubella cases and outbreaks;
- Insufficient information provided together with clinical samples;
- Maintain high vaccination coverage;
- Educate health care workers about their possible role in spreading viruses if they are not properly immunized;
- Convince health care workers to get properly vaccinated;
- Objection to vaccination in certain population groups;
- Difficulties to reach certain population groups for vaccination programs;
- Rapid staff turn-over;
- Lack of experienced, well-trained staff;
- Improve collaboration between laboratory and epidemiologists;
- Communication with subnational laboratories;
- Determine the extent of deep-pockets of susceptibility in hard-to-serve population groups;
- Access to diagnostic kits, reagents, and other consumables;
- Intensify collaboration with clinicians;
- Countries or areas with much tourism may experience imported cases of measles and rubella;
- Current economic crises may affect vaccination programs and elimination efforts;
- Surveillance for congenital rubella syndrome;
- Establishing a differential diagnosis in measles- and rubella-negative suspected cases;

Discussion

- For an accurate laboratory diagnosis of SSPE, serum and CSF need to be tested simultaneously for measles-specific antibodies with albumin used as a control antigen. Very high measles IgG antibodies in serum and in CSF (determined by titration of the samples), and also very high titers in complement fixation assays is a usual finding. The CSF/serum IgG index as well as the detection of MV-specific oligoclonal bands in CSF should be determined in a highly specialized lab; interpreting the measurement values requires particular experience. IgM serology is not appropriate for diagnosis, because it is always negative. Measles RNA is not detectable in CSF by PCR. Some cases with a positive PCR finding from brain biopsy material have been described.
- The Philippines are experiencing large measles outbreaks and several genotypes are circulating in the country. Many imported cases in EUR originate from the Philippines. Among those, genotype B3 has been

circulating for a long time and its spread might be due to mass gatherings, but firm epidemiological data is not available.

- Infection in vaccinated individuals leads to low or lacking IgM response. However, France has regularly observed long-lasting rubella-specific IgM antibodies following rubella vaccination.
- Rubella IgM testing should not be systematically done during pregnancy. Such tests are only indicated if symptoms compatible with rubella case definition are observed.
- National Reference Laboratories should take on an oversight role over other laboratories performing measles and rubella diagnosis in the country to comply with verification requirements. Depending on available resources, they might even arrange some national EQA program.
- Updating on a relevant Measles Rubella laboratory manual is under progress.

SESSION 4: MEASLES RUBELLA LABORATORY INVESTIGATION AND ACCREDITATION ISSUES

Chair Irja Davidkin (THL, Helsinki)

5.2 *Measles Rubella diagnostic approach in vaccinated persons* – Sabine Santibanez (RKI, Berlin)

In order to establish a diagnosis in vaccinated patients with clinical signs of measles, detection of viral RNA needs to be included, besides serological tests. Samples for RNA detection must be collected during the first days of illness. In case the patient has been vaccinated less than 21 days before onset of symptoms, genotyping helps differentiating between vaccine-associated measles (genotype A) and wild-type measles (any other genotype). If the patient has received vaccination more than 21 days before onset of symptoms, differentiation can be made between primary and secondary vaccine failure. In primary vaccine failure, the patient's symptoms meet case definition and laboratory data indicate primary infection, i.e., a primary immune response with IgG seroconversion and IgM antibodies. In cases of secondary vaccine failure, the patient's symptoms may at least partly meet clinical case definitions. Most patients with secondary vaccine failure present with fever and headache; often the rash is not generalized, conjunctivitis, cough, or rhinitis may be absent. Laboratory data indicate reinfection, i.e., IgG antibodies are present already in the acute-phase serum and the IgM response may be absent or only mild. The avidity index of measles-specific IgG antibodies is low (but increasing over a period of 7 to 8 weeks) in primary infection, and high in reinfection. A defect in antibody affinity maturation may contribute to vaccine failure. In cases of vaccine failure it is important to obtain specific information, in order to enable correct interpretation of laboratory results. This information should include date of birth, documentation of vaccination (dates of

first and second vaccination, vaccine used), date of symptom onset and description of symptoms, date of sample collection, possible contacts, and whether the patient is a health care worker.

5.2 Update on WHO Measles-Rubella molecular Program – Mick Mulders (WHO headquarters)

The Molecular external quality assurance (mEQA) program is mandatory for all RRLs, and for NLs performing molecular testing for measles and rubella in 2015. Participating laboratories are encouraged to use their routine techniques, their own standard reagents and SOPs when testing the mEQA panels. Measles practice panels have been dispatched in 2012. Real-time PCR protocols appear to work well on different platforms. However, some laboratories have experienced cross-contamination problems. Genotyping of RT-PCR products works well, but some chromatograms obtained were of low quality. Also rubella FTA card practice panels have been dispatched in 2012. Most laboratories performed well, correctly identified the genotypes, and reported their results in a timely manner. The reporting format, though, needs to be improved. Scoring criteria for the mEQA will include the correct detection of measles and rubella RNA by RT-PCR; production of required PCR amplicons for genotype analysis; correct identification of the genotype base on sequence analysis.

5.2 Laboratory reporting – Myriam Ben Mamou (WHO/Europe, Copenhagen)

The 28 EU countries with Iceland and Norway report measles and rubella data to TESSy and 23 other countries report directly to WHO. All data is compiled in the Centralized Information System for Infectious Diseases (CISID). As of January 2014, 51 Member States collect case-based data and 42 Member States report case-based measles data to WHO. The corresponding numbers for rubella case-based data reporting are 47 and 34, respectively. Timeliness and completeness of data reporting needs to be improved in order to reach the WHO/Europe reporting target of >80%. Aggregated data is presented monthly on the WHO/Europe website. Technical improvements are planned for the online Measles Rubella Laboratory Data Management System (MRLDMS; <http://mrlcms.euro.who.int>) and users are encouraged to provide input. This specimen-based system is expected to improve surveillance data quality, as it will provide better linkage between case-based reporting (CISID) and laboratory data. Laboratories should record specimen details and test results in the MRLMS immediately upon arrival of specimen to the laboratory and when test results become available. In 2014, 26 laboratories have registered 4,470 specimens in the MRLDMS. At country level, national stakeholders are encouraged to continue efforts towards getting a unique identifier to facilitate case-base surveillance and data analysis.

Discussion

- The question was raised whether individuals with vaccine failure have been vaccinated with inactivated or live vaccine. For the recent measles outbreak in Slovenia it is known that people with vaccine failure have received live vaccines.
- It is important to understand how much vaccine failure cases affect elimination programs and therefore as much information as possible should be collected. It is unlikely that vaccine failure cases contribute much to measles transmission, at least not in the open population. Vaccine failure in health care workers, though, may lead to transmission chains.
- It is unlikely that wild-type measles viruses could escape neutralization by vaccine-induced antibodies.

5.2 *Check-list issues and questions from the NRLs – Irja Davidkin (THL, Helsinki)*

Laboratories are encouraged to provide feedback from check-lists. Responses differ between laboratories. Performance of internal laboratory controls should be provided as graphical output, which would assist comparisons between laboratories. It is important to monitor for drift in test performance when new internal standards are taken in use. This cannot be achieved if moving averages are used. For laboratories with large number of cases, data entry presents major workload.

SESSION 5: UPDATE ON WHO MEASLES AND RUBELLA GENETIC SURVEILLANCE DATABASES

Chair: Kevin Brown (WHO GSL, London)

5.1 *Introduction to MeaNS and RubeNS databases – Kevin Brown (WHO Measles/Rubella Global Specialized Laboratory, London)*

The MeaNS database (www.who-measles.org) is intended for storing and analysing measles sequences. The database provides tools for genotyping of viruses and for phylogenetic analyses. Information from the MeaNS database can be directly reported to WHO, and sequence data can, with the permission of the originating laboratory, be uploaded to GenBank. Through these mechanisms multiple reporting can be avoided. As of early May 2014, the database contains records of approximately 15,500 clinical samples and more than 16,000 measles gene sequences. Most sequences submitted so far are from the WHO European Region. MeaNS will generate a name for the virus according to the convention published in the Weekly Epidemiological Record (WER 2012; vol. 87(9):73-80) if sufficient information is provided to MeaNS. Currently sequences need to be uploaded in ASCII mode. The website provides a FAQ section and an online training video:

(http://www.hpa-bioinformatics.org.uk/richard/Measles/Public/Web_Front/main.php).

The RubeNS database is currently located at the URL: <http://www.hpa-bioinformatics.org.uk/rubella> but will soon move to: www.who-rubella.org. As of early May 2014, this database contains just over 1,000 rubella virus sequences. Although the RubeNS platform looks different from the MeaNS platform, it provides basically similar possibilities.

5.2 Practical session: Using MeaNS and RubeNS – Richard Myers (remotely through video connection; WHO Measles/Rubella Global Specialized Laboratory, London)

Richard Myers demonstrated the use of the two databases and explained the different functions and tools available. For registration to the databases, a quick web-form must be completed. A phylogeography options allows to monitor what viruses are circulating in what geographic areas and when.

3. CONCLUSIONS & RECOMMENDATIONS

Recommendation 1: POLITICAL COMMITMENT and ACCOUNTABILITY

Mobilize countries' and partners' political commitment and accountability for maintaining a proficient and sustainable measles and rubella laboratory network

The WHO European Measles and Rubella Laboratory Network (LabNet) is strong and proficient, has good expertise and is able to deal with the current workload. However, with incidence of measles and rubella decreasing on the way towards elimination, national reference laboratories (NRLs) are facing new demands and responsibilities.

- 1.1. Countries need to increase ownership and investment in their national reference laboratories to ensure continued and sustainable ability to deal with increasing workload, including additional testing and enhanced molecular surveillance.**
- 1.2. Partners should increase support to LabNet to enable it to meet the demands of testing in low-incidence settings and verification of elimination.**
- 1.3. The WHO Regional Office for Europe (Regional Office) is requested to share officially the recommendations of the present meeting with the respective ministries of health to foster increased support for their respective measles/rubella national laboratories and to enable adequate conditions and sustainable funding to perform their duties.**

Recommendation 2: CASE-BASED SURVEILLANCE and LAB-EPI LINKAGE

Improve measles/rubella laboratory and epidemiological data integration and strengthen case-based reporting as a key contribution to verifying measles and rubella elimination

Case-based surveillance with laboratory confirmation is critical to monitor the progress of the measles/rubella elimination programme. Appropriate linkage of epidemiological and laboratory data is key for timely classification of cases, detection and response to outbreaks, assessment of country status (verification) and, overall, to inform decision-making.

- 2.1. As an essential component of the measles and rubella elimination programme, countries must maintain a high-quality surveillance system for measles, rubella and desirably also for congenital rubella syndrome.**
- 2.2. Laboratory data must be assessed together with field epidemiological data and reviewed regularly at the national level. Countries should advocate for a unique identifier at national level in order to facilitate data collection and analysis.**
- 2.3. The Regional Office developed a specimen-based tool to collect measles/rubella laboratory investigation data (MRLDMS). Network laboratories are encouraged to implement the MRLDMS tool. In consultation with LabNet, the Regional Office needs to upgrade MRLDMS with additional functionalities to allow more flexibility, usefulness and buy-in by the network.**
- 2.4. The Regional Office and the European Centre for Disease Prevention and Control (ECDC) should continue their ongoing collaboration to coordinate and optimize the reporting via the European Surveillance System (TESSy) and Centralized Information System for Infectious Diseases (CISID).**

Recommendation 3: FULL ACCREDITATION of REFERENCE LABORATORIES

Comply with LabNet standards and maintain sufficient performance enabling full accreditation and contribution to the verification of measles/rubella elimination

A fully accredited national laboratory according to current WHO laboratory network standards is an essential condition to provide high-quality information that will contribute to documenting the achievement of measles and rubella elimination.

- 3.1. All laboratories should participate in the assessment for full accreditation and comply with all criteria as appropriate, including timeliness and completeness of lab reporting to WHO. In case of non-conformity, accreditation may be provisional or cancelled.**
- 3.2. In coordination with regional reference laboratories (RRLs), national laboratories should submit samples for confirmatory testing originating only from suspect cases and accompanied with complete data (origin of samples, laboratory investigation data and final conclusion reported). Use of filter papers may facilitate shipment.**
- 3.3. LabNet laboratories should actively participate in outbreak investigation. If genotyping cannot be performed in the laboratory, PCR products or appropriate clinical specimens should be submitted timely to the RRL. Use of filter paper for analysis of nucleic acids (FTA cards) overcomes cold chain,**

biosafety and financial issues. Samples should be sent with adequate information about the patient, vaccination status and the epidemiological situation.

- 3.4. RRLs and NRLs performing routine measles/rubella molecular testing of clinical specimens are required to participate in the new LabNet programme for molecular external quality assessment (EQA).**
- 3.5. It is expected that NRLs establish an oversight mechanism over the subnational laboratories which perform measles and rubella testing, including a national EQA programme based on LabNet standards.**
- 3.6. For surveillance, it is not recommended to perform systematic screening with IgM testing in absence of suspected case (case definition).**
- 3.7. In collaboration with RRLs, national laboratories should use a comprehensive approach including additional laboratory confirmatory methods to confirm measles infection in vaccinated individuals, as described in surveillance guidelines. Publication of evidence-based data on the role of vaccines in measles transmission to contacts is encouraged.**

Recommendation 4: MOLECULAR SURVEILLANCE

Enhance molecular surveillance of measles and rubella viral sequences and scale up the reporting to MeaNS and RubeNS databases

In the Framework for the verification process in the WHO European Region, genotyping evidence is one of the three essential criteria supporting the interruption of endemic transmission. The programme needs to place increased emphasis on collection of appropriate samples for virologic surveillance and timely reporting of molecular epidemiology data, in order to improve the knowledge of molecular epidemiology both for measles and rubella.

- 4.1. Laboratories are requested to genotype at least 80% of the number of epidemiologically identified transmission chains and timely report sequence data to their national surveillance system and within two months to the WHO genetic surveillance databases MeaNS and RubeNS.**
- 4.2. As recommended earlier, to identify the genotype of a measles virus it is necessary and sufficient to sequence the 150 amino acid sequence from the carboxy-terminal end of the measles virus N gene.**
- 4.3. NRLs that do not have the capacity to perform routine measles/rubella sequencing are requested to send appropriate clinical samples or PCR amplicons for genotyping to their respective RRLs. The use of FTA classic cards simplifies shipment.**
- 4.4. Laboratories that do not yet have access to MeaNS and RubeNS databases are invited to register online to submit their sequence data and perform**

sequence analysis, in strict observance of terms and conditions of use of the databases.

- 4.5. LabNet needs to advocate at all level to increase molecular surveillance and reporting and provide feedback to national laboratories on newly submitted sequences.**
- 4.6. LabNet should renew the programmatic and laboratory emphasis on laboratory investigation of rubella suspected cases and rubella virus genotyping.**

Annex 1: Abbreviations

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| AFRO | World Health Organization Regional Office for Africa |
| CISID | Centralized information system for infectious diseases |
| ECDC | The European Centre for Disease Prevention and Control |
| EEA | European Economic Area |
| ELISA | Enzyme linked immunosorbent assay |
| EMRO | World Health Organization Regional Office for Eastern Mediterranean |
| EQA | External Quality Assessment |
| EU | European Union |
| EUR | WHO European Region |
| GSL | Global specialized laboratory |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| Labnet | Laboratory network |
| MeaNS | Measles Nucleotide Surveillance |
| MRLDMS | Measles and rubella laboratory data management system |
| NRL | National reference laboratory |
| OF | Oral fluids |
| PCR | Polymerase chain reaction |
| PHE | Public Health England |
| RKI | Robert Koch Institute |
| RNA | Ribonucleic acid |
| RRL | Regional reference laboratory |
| RubeNS | Rubella Nucleotide Surveillance |
| SEARO | World Health Organization Regional Office for South-East Asia |
| SIAs | Supplementary immunization activities |
| Tessy | The European Surveillance System |
| US | United States |
| WHO | World Health Organization |
| WHO/Europe | World Health Organization Regional Office for Europe |
| WHO headquarters | World Health Organization Headquarters |