



**World Health  
Organization**

REGIONAL OFFICE FOR

**Europe**

**9th Meeting of the  
Measles/Rubella  
Reference Laboratories of  
the WHO European Region  
10–11 March 2014, Copenhagen,  
Denmark**

**Meeting report**



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## Abbreviations

B19V	Parvovirus B19
BLAST	Basic Local Alignment Search Tool
CDC	United States Centers for Disease Control and Prevention
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
ES	enhanced (active) surveillance
EQA	External Quality Assessment
EU	European Union
GSL	Global specialized laboratory
HH6	Human herpesvirus type 6
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Labnet	Laboratory network
MeaNS	Measles Nucleotide Surveillance
MRLDMS	Measles and rubella laboratory data management system
NGS	Next Generation Sequencing
NIS	Newly independent states
NL	National laboratory
NRL	National reference laboratory
OF	Oral fluids
PAHO	Pan-American Health Organization
PCR	Polymerase chain reaction
PHE	Public Health England
PRN	Plaque reduction neutralization
PP	Proficiency panel
RAGIDA	Risk assessment guidelines for infectious diseases transmitted on aircraft
RKI	Robert Koch Institute
RNA	Ribonucleic acid
RRL	Regional reference laboratory
RubeNS	Rubella Nucleotide Surveillance
RVC	Regional Verification Commission
SAGE	The Strategic Advisory Group of Experts on Immunization
Tessy	The European Surveillance System

## Executive summary

The WHO Regional Office for Europe convened a technical meeting in Copenhagen, Denmark on 10–11 March 2014 to review the status and performance of the European Measles Rubella Laboratory Network (Labnet), particularly in the context of approaching measles and rubella elimination and to address particular issues related to accreditation, contribution of Labnet to the verification process, molecular epidemiology, molecular external quality assessment and seroprevalence studies.

The meeting brought together representatives from the European measles/rubella regional reference laboratories (RRLs) in Germany, Luxembourg and the Russian Federation, the Global Specialized Laboratory (GSL) in the United Kingdom, the United States Centers for Disease Control and Prevention (CDC), the European Centre for Disease Prevention and Control (ECDC), WHO headquarters and the Regional Office for Europe.

Progress, challenges and lessons learnt were reviewed during the meeting, and solutions and future plans were discussed.

The following key recommendations were made.

1. Strengthen case-based reporting as a key contribution to verifying measles and rubella elimination.
2. Follow up on accreditation issues and provide tailored technical advice to national reference laboratories to comply with WHO Labnet standards.
3. Enhance molecular surveillance of measles and rubella viral sequences and scale up the reporting to the global WHO databases for measles and rubella nucleotide surveillance (MeaNS and RubeNS).
4. Develop technical guidance for the next steps of measles and rubella elimination in the WHO European Region.

## Introduction

The WHO Regional Office for Europe coordinates the European Measles and Rubella Laboratory Network (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.

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.

The Regional Office convened a technical meeting in Copenhagen, Denmark on 10–11 March 2014 to review the status and performance of Labnet. The scope of the meeting was to:

- provide an update on Labnet in the WHO European Region and a platform for participants to exchange information on recent developments and challenges from the operational and research and development perspectives;
- discuss Labnet's contribution to measles and rubella reporting/surveillance, especially in the context of verification of elimination of the two diseases in the European Region;
- address particular issues related to accreditation, molecular epidemiology, molecular external quality assessment and seroprevalence studies;
- provide an opportunity to host side meetings of the steering committees of the global WHO databases for measles and rubella nucleotide surveillance (MeaNS and RubeNS).

The meeting brought together representatives from the European measles/rubella regional reference laboratories (RRLs) in Germany, the Russian Federation and Luxembourg, the Global Specialized Laboratory (GSL) in the United Kingdom, the United States Centers for Disease Control and Prevention (CDC), the European Centre for Disease Prevention and Control (ECDC) and WHO headquarters and the Regional Office for Europe. Apologies were received from Professor C. Muller (Luxembourg) and Richard Myers (GSL).

The purpose of the meeting was to decide about concrete recommendations and priority actions to be taken in the areas of reporting, coordination with ECDC, operational aspects of sample collection and transportation at national levels, algorithms of laboratory confirmation in elimination settings, sequencing strategies and genotyping, accreditation, capacity building, seroprevalence studies, funding and research and development.

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.

## **Sessions**

Dr Guenael Rodier, Director of the Division of Communicable Diseases, Health Security and Environment at the WHO Regional Office for Europe opened the meeting by welcoming all participants and giving a short overview of the measles and rubella situation. He stressed the important role of Labnet for the verification of disease elimination and thanked all the participants for their continuous efforts and contributions.

### **Session 1: Measles/rubella programme and Labnet updates**

Chair: Dr Paul Rota

#### ***1.1 WHO updates***

##### **1.1.1 Global update**

Dr Mick Mulders updated the participants about the Labnet on a global scale. He talked about the WHO African Region and new measles elimination goals for the WHO South-east Asia Region and warned that global vaccination coverage is levelling off and that it will be very hard to reach the goals. This is especially the case in the African Region but also some countries in



the European Region do not show any progress since 2009 in measles-containing vaccine dose 1 (MCV-1) coverage, and overall the incidence and mortality rates show a too slow decrease. For rubella a slow improvement in vaccination coverage has been observed, but much more comprehensive surveillance data are needed.

There are currently 694 laboratories in the Labnet with additional subnational labs being established in Ethiopia, Indonesia and Thailand. In the European Region there are currently 52 national laboratories (NLs) in 47 Member States (Bosnia and Herzegovina, France and the Netherlands have two each), 1 GSL, 3 RRLs and 20 subnational labs.

Especially in the African Region a large proportion of the cases is only epi-linked and the laboratory confirmation rate is still low. In the past year the European Region had the highest testing workload for measles, while for rubella the Eastern Mediterranean Region tested most samples. The most prevalent genotypes lately were D8 and B3 and it seems that B3 is most prevalent so far in 2014. Dr Mulders emphasized the need to collect specimens for genotyping, especially for rubella, and for the sequence to be submitted to the relevant databases.

New accreditation checklists are now available for both NLs and RRLs. One aim of the updated lists is to directly provide information needed by the Regional Verification Committee for the certification of measles and rubella elimination (RVC). The meeting participants were encouraged to check whether this aim is being reached with the current versions of the checklists; if not, suggestions for improvement are welcome.

Concerning the molecular external quality assurance (EQA) panel, the distribution is slightly behind schedule. New molecular training workshops are planned and guidelines for seroprevalence studies are being prepared based on current guidelines for hepatitis B virus.

Among the challenges mentioned by Dr Mulders is the already high workload of many laboratories, the need for enhanced surveillance in countries introducing rubella-containing vaccine, the need for additional laboratory testing in settings with improved disease control, staff turnover, especially in the African Region and funding issues. Dr Mulders stressed that the role of Labnet becomes increasingly important with improving disease control.

During the discussion after the presentation, it was suggested that the timeframe of reporting in the accreditation checklist should be synchronized with the country reports needed for the RVC in order to avoid preparing two different reports. There may be different requirements for laboratories in the elimination phase compared to settings where elimination is to be maintained.

### 1.1.2 European regional update

Dr Myriam Ben Mamou showed that the European Region has been declared off-track by SAGE concerning the 2015 elimination goals. There were more than 30 000 measles cases in the Region in 2013, mainly in young adults and in unvaccinated people or in persons with unknown vaccination status. The Region still has many cases that are not laboratory confirmed and this is not acceptable given the current stage of disease control. There are still large outbreaks, but very few sequences are submitted and many countries (10) with cases do not have any genotype data. In The European Region D8 was also predominant in 2013 and now B3 seems to be taking over. Almost 40 000 rubella cases were reported in 2013 with the large majority coming from Poland. Very few cases were laboratory confirmed and most were just clinically confirmed. Several

countries still do not provide comprehensive data on rubella and especially sequence information is lacking.

In the European Region nearly 60 000 specimens were collected in 2013, most of them being serum samples and in the western part also oral fluid (OF). In recent years there has been nearly no improvement in the performance indicators for measles and rubella. About 53 000 specimens were analysed for measles IgM antibodies and 25 000 for rubella antibodies.

The results of the last proficiency panel (PP) were quite good, which may be because it was not so difficult. For 2014 all but four labs were fully accredited. The accreditation for the lab in Tajikistan is pending; the labs in Banja Luka in Bosnia and Herzegovina, the former Yugoslav Republic of Macedonia and Turkmenistan were provisionally accredited. Among the challenges for the European Labnet identified by Dr Ben Mamou were the linkage between laboratory and epidemiological data, the low laboratory investigation rate and lacking genotype baseline data for rubella, budget limitations and the continuing outbreaks in the context of the 2015 elimination goals. Several meetings and workshops are planned to address some of the problems.

Participants asked whether more NLs should get support and training for molecular testing or the referral of samples to the respective RRL should be promoted. There was agreement that in the future the NLs must be able to do additional testing, also including molecular testing and that for now NLs with an existing infrastructure should be encouraged to implement molecular testing for measles and rubella. This may be especially relevant for countries with low case numbers. It was suggested that if necessary national recommendations should be modified to include the collection of specimens for molecular investigations directly at first presentation of the patient. The question was also raised of why specimens are collected for measles but not for rubella although the same types of specimens are required. Especially for CRS cases the question was whether Labnet is disconnected from specimens collected or whether the infectivity of CRS cases is not monitored at all. The lack of rubella genotype data may be due to difficulties in obtaining the 739 base pairs genotyping region directly from clinical specimens and the comparatively short window for rubella virus detection. In OF this may be as short as 2-3 days after rash onset for rubella, while for measles it may be up to one month. There was a suggestion to try and change national guidelines to better investigate rubella cases.

## ***1.2 Reference laboratories updates***

### **1.2.1 Global specialized laboratory update (London, England)**

Dr Kevin Brown informed the participants that since the re-structuring of the institution, Northern Ireland, Scotland and Wales have been acting independently, but reporting is still done through Public Health England (PHE). Measles containing vaccine (MVC) coverage rates are still increasing, although there are differences within the country and outbreaks were reported from the United Kingdom and Wales. As a consequence of the Wakefield incident<sup>1</sup>, and concerns about vaccine status, measles is now being seen in older age groups. There was a dramatic drop in case numbers after catch-up campaigns in England and Wales. In 2013 D8 dominated and there were two main lineages. For rubella virtually all cases were associated with importation. The confirmatory testing for Northern Ireland yielded only 31 out of 37 concordant measles OF results. This may be due to a storage problem in the NL; in addition they do not

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<sup>1</sup> In 1998, the Lancet published a paper authored by A. Wakefield implying a link between MMR vaccine and autism in children. Several studies later invalidated this hypothesis, fraud in Wakefield work was proved and the Lancet retracted the initial paper. Despite this, the "Wakefield incident" was a major cause of MMR vaccine hesitancy, particularly in the UK.

check the total IgG level to assess the quality of the OF sample before IgM testing. There have been problems with the supply of Microimmune rubella IgG kits since a new company took over production; and currently this product is not available, leading to major problems for PHE in testing OF samples. As a consequence PHE is developing a new in-house assay for rubella IgG testing. In addition it has established a new triplex PCR targeting the measles H and N gene and an internal control. Also the point-of-care test development coordinated by Dr David Brown is ongoing. The question was raised of whether the measles and rubella strain banks should be maintained. No isolates have been submitted for several years now.

### 1.2.2 Regional laboratory updates

#### Koch Institute (Berlin)

Dr Annette Mankertz reported that the Institute recently moved to a new building and achieved national accreditation. The Institute has seen several different ministers of health, a new head of department and will have a new president next May and with each change in staff they have to justify again the importance of the work they do. She reported that Sweden had problems with the last PP and she sees a clear need for basic training in serology. The NL in Italy is very difficult to get in touch with as large re-organizations are ongoing. They have several NLs who receive very few samples, often because private labs are doing measles and rubella testing. Some labs send samples both from screening programmes and from suspected cases. Not all countries submit their sequence data to MeaNS and Dr Mankertz therefore suggested that NLs should be informed about the video on how to submit sequences to MeaNS. There are still many cases in Germany, mainly caused by genotype D8 viruses. In recent years an increasing number of reinfections has been observed based on IgG avidity and PCR results and on vaccination records. Due to data protection issues case-based reporting is not possible and some other countries have similar constraints. The challenges faced by the RRL Berlin include lack of or late notification about outbreaks in its constituency and an increasing number of vaccine objectors. The recently implemented rubella notification policy does not yet work properly.

#### Laboratoire national de santé (Luxembourg)

Dr Judith Hübschen reported that two labs in its constituency (Banja Luka in Bosnia and Herzegovina and in the former Yugoslav Republic of Macedonia) were only provisionally accredited for 2014. The RRL Luxembourg provided an increasing number of filter papers to the NLs for sample shipment for confirmatory testing. The results of the last PP were very good and all labs had a score of 100% for measles and all also passed for rubella with at least 90%. For confirmatory testing several NLs that received a low number of samples and the lab in Banja Luka did not receive a single specimen for measles or rubella testing in 2013. The workload slightly decreased in 2013 compared to 2012 and the large majority of the samples received were liquid followed by dried serum. The diversity of ELISA kits used is slightly decreasing with Siemens kits being largely predominant. During the last confirmatory testing session at the beginning of 2013, two labs (the former Yugoslav Republic of Macedonia and Turkey) failed for measles and one of them (the former Yugoslav Republic of Macedonia) also for rubella. Measles sequence data were obtained from three countries within the constituency and two countries in the framework of collaborations.

#### Gabrichevsky Institute (Moscow)

Dr Tamara Mamaeva provided an overview of PP testing in the RRL Moscow constituency and confirmatory testing done in their lab. Tajikistan did not participate in the PP testing due to re-

organizations in the country and Turkmenistan did not send any samples for confirmatory testing. Two labs (Tajikistan and Uzbekistan) sent fewer than 50 samples. The same samples are used for measles and rubella confirmatory testing. The subnational labs showed a good performance. In some countries a high percentage of sera were collected less than four days after rash onset and efforts to change this during the past years did not work. A total of 25 suspected rubella cases that were tested IgM positive in private labs were not confirmed in the RRL Moscow, underlying the importance of using registered quality reagents. In the Russian Federation nearly 76% of the suspected measles cases with samples were laboratory confirmed, while only a small percentage of rubella-suspected cases with samples were confirmed in the laboratory. Among nearly 2800 rash/fever samples 129 measles IgM positives were identified. Thirty-one of them were discarded as measles cases due to a high avidity and a low and not increasing IgG titer. The RRL Moscow did a comparison between the new version of the Euroimmun kit registered since 2012 and the previous version and other kits including Siemens and found that the new Euroimmun kit shows results similar to Siemens. During an outbreak in the Russian Federation many people between 20 and 35 years of age were affected. They were IgM positive, had high avidity and a high IgG titer. In her conclusions Dr Mamaeva pointed out that:

- some countries do not always have test kits available and this may lead to late testing and reporting,
- in the event Euroimmun kits are used for confirmatory testing there is a need to develop a protocol for elution of serum from filter paper; and
- a workshop is needed before the MRLDMS system for reporting is introduced in the constituency.

In the subsequent discussion it was clarified that measles transmission from cases with vaccine failure was confirmed by PCR. It was suggested that to confirm rubella in pregnancy, PCR testing and at later time points IgG avidity testing should be performed in addition to IgM testing. In the ECDC/EU case definition for rubella, when rubella in pregnancy is suspected, further confirmation of a positive rubella IgM result is required (e.g. rubella-specific IgG avidity test showing a low avidity). In the United States, where rubella elimination has been achieved, the recommendation is that rubella IgM testing should not be done at all during pregnancy. In the Russian Federation there is a guideline that at first presentation specimens for PCR and genotyping should be collected, and between four and seven days after rash onset a sample for serology should be taken.

## **Session 2: Verifying measles and rubella elimination**

Chair: Dr Annette Mankertz

### ***2.1. 2014 Regional Measles and Rubella Verification Framework***

Dr Sergei Deshevoi presented the current status of the review of reports from the European Region national verification committees (NVCs) by the Regional Verification Commission for Measles and Rubella Elimination (RVC). Twelve countries had not submitted reports and three reports were not considered because they were not submitted by the NVC of the country. Thirty-three reports in total were evaluated based on the different lines of evidence (population immunity, epidemiology and genotype data, surveillance performance, sustainability of immunization programme and supplementary evidence). Fifteen countries were classified as

having interrupted endemic measles virus circulation and 18 countries as having interrupted endemic rubella virus circulation. The main challenges were the quality, consistency and completeness of data. The lowest response rates were obtained for immunization coverage, genotype information and public acceptance of vaccination. He emphasized that essential criteria to demonstrate interruption of endemic transmission are the absence of endemic measles and rubella cases, high quality surveillance data and genotype information. Alternative surveillance performance indicators comprising the timeliness of notification and the rate of cases tested negative for measles and rubella IgM antibodies were presented. As far as the supporting lines of evidence are concerned, Labnet plays a prominent role to provide data for molecular epidemiology of measles and rubella viruses. In the subsequent discussion the definition of the different control status terms was clarified. Revised elimination criteria/definition and new forms will be published shortly. The United States experience has now been published (Papania MJ et al, JAMA Paediatr 2014 Feb;168(2):148-55).

## ***2.2. Measles and rubella case-based surveillance and reporting***

Dr Myriam Ben Mamou gave an overview of current reporting practice in the WHO European Region and the potential role of the MRLDMS database. She explained that for measles 51 Member States collect case-based data and 42 of them provide data to WHO. For rubella 47 countries collect case-based data and 34 report to WHO. The timeliness and completeness of reporting in the Region is not optimal. Several countries did not provide any report in 2013. The idea of MRLDMS was to obtain complementary reporting of the Member States and the corresponding NLS. One of the current problems is that there is a difference between the numbers reported by the countries (laboratory-confirmed cases) and by the laboratories (laboratory-tested specimens), which is further complicated by the testing done in private laboratories. Another problem is that in the framework of screening programmes IgM testing is done for specimens that are not from suspected cases. Challenges for a more widespread use of the MRLDMS system within the European Labnet are the currently very high workload to enter individual case data, the need for case-based data and for a technical upgrade of the database. During the discussion it was specified that MRLDMS is a specimen-based database and not case based. While the aim of the database is to synchronize national data, the system may not be flexible enough and not all data are recorded. The Labnet participants agreed that changes are required to make it a useful tool for them.

## ***2.3 Surveillance of measles and rubella in the European Union (EU) and reporting to TESSy/CISID***

Dr Sabrina Bacci introduced the contributions of ECDC to WHO aims and the current reporting practice via the case-based Tessy system. At present 30 EU/European Economic Area countries report measles data and 27 report rubella data via TESSy. Several countries have an automated system for reporting and may update their data to TESSy basically every day. Every month, ECDC shares the data with the WHO Regional Office for Europe, which incorporates the data into CISID. The reporting to TESSy by the EU countries occurs only for the so-called "Member States" reporting and does not aim at collecting the variables included in the CISID laboratory aggregate reporting as well as the variables collected by MRLDMS. In the current incidence maps prepared by ECDC there is no discrimination according to the importation status. In different countries different age groups are most affected by measles, but overall many cases are observed among children less than one year of age and among adolescents and adults. The question was raised of whether additional laboratory performance indicators should be incorporated in Tessy and/or CISID, but this was not considered necessary as the currently collected case-based data are sufficient to determine these indicators. The possible reasons for

the unusual age distribution among measles cases in Romania were discussed. The ideal reporting scenario would be to exchange and synchronize surveillance data obtained at different levels within a country, use the same national identifier for both the “Member States” reporting and the MRLDMS, and then report the data to WHO (or to ECDC and then WHO for the EU Member States). A likely explanation of why the epi-identifier system works for polio but not yet for measles may be that there are by far fewer polio-suspected cases and they are all investigated in public health reference laboratories.

### **Session 3: Seroprevalence studies**

Chair: Dr Joe Icenogle

#### ***3.1. WHO headquarters perspective – global guidelines***

Dr Mick Mulders presented the essential criteria for measles and rubella elimination. Among the lines of evidence ranges an assessment of population immunity including marginalized groups. Seroprevalence studies may provide an additional means to validate immunization coverage estimates, to generate population susceptibility profiles, to identify gaps in immunization coverage and high-risk groups. It is, however, very important to know before you design such a study, exactly what question you are trying to answer. In addition, many of these studies have serious shortcomings, for example concerning statistical analysis, sample size, collection and testing of samples or the conclusions drawn from the results. Global guidelines for seroprevalence studies for measles and rubella are currently being developed by WHO based on existing guidelines for hepatitis B virus. The steps and timeline for the development of the guidelines were presented. In the subsequent discussion Dr Icenogle stressed that positivity for IgG antibodies does not necessarily mean immunity. For some people with very low levels of antibodies for rubella, this value may rise above the threshold shortly after vaccination and then fall back to low levels again. This makes the timing of such a study critical: shortly after a vaccination campaign the results may be different from what is seen five years later. It was also suggested that based on the results of seroprevalence studies, changes in vaccination schedules may be made, but it is not planned to add any such recommendations to the seroprevalence study guidelines.

#### ***3.2 Highlights from research and development studies***

Dr Judith Hübschen presented an overview of recently completed and currently ongoing research projects relating to measles and rubella at the RRL Luxembourg. Among the projects were outbreak investigations, seroprevalence studies, rash/fever disease and CRS investigations, measles complete genome amplicon sequencing using next-generation sequencing (NGS) technology and more basic research investigations. The likely shortcomings of the CRS study were discussed and the question was raised of how far seroprevalence studies targeting Roma communities are considered useful.

#### ***3.3 Experience of the Russian Federation and newly independent states***

Dr Galina Lipskaya showed that after several years with very low measles incidence in the Russian Federation, case numbers have been rising again since 2011. Most territories still have low incidence levels and only few regions have very high rates. An analysis of who is currently affected by measles in the Russian Federation showed that many patients were unvaccinated or vaccinated a long time ago. There are many cases among adults. Risk groups in the country comprise hard-to-reach populations, medical staff, staff of educational institutions, students and

salespersons. Lately the Russian Federation also faced an outbreak in a religious community that objects to vaccination, and overall the numbers vaccine objectors and hard-to-reach groups have increased. Another problem is that diagnosis is not always made very quickly and thus outbreak countermeasures are delayed. Advocacy to promote vaccination is needed as most cases occur in unvaccinated people or persons with unknown vaccination status. So far most cases have not initiated outbreaks.

## **Session “hors categorie”: MeaNS – RubeNS steering committees**

Chair: Dr Kevin Brown

Please see separate minutes of the session.

## **Session 4: Laboratory confirmation in the context of elimination**

Chair: Dr Mick Mulders

### ***4.1 Issues and challenges with the current serology approach***

Dr Judith Hübschen presented some of the problems that the RRLs are currently facing during confirmatory testing, including IgM positive samples obtained in the frame of screening programmes of asymptomatic people, a comparatively high rate of false positives due to good disease control and a final case classification based on other data than IgM testing or IgM testing alone. The confirmatory testing of these samples results in more major discrepancies. Additional laboratory investigations may be needed to try and resolve these discrepancies; and for the scoring of the results also the final case classification of the NLs may need to be taken into account. In addition some assays seem to be more prone to yield weak and false positive results. A few examples of test results obtained with different kits and lot numbers were shown and for samples with corrected delta optical density (OD) values close to cut-off values the qualitative results obtained with different test systems may easily vary between “negative” and “positive”. If different test systems are being used and also different types of assays, such as IgM tests, IgG tests, IgG avidity tests or PCR, it may be necessary to provide for each discrepant result a statement about the most likely explanation and interpretation of the results. For WHO accreditation a score is needed, and this evaluation needs to be translated into a percentage of concordance. It was suggested that NLs should add into a new column their final case classification, and the samples referred to the RRLs to obtain help with the interpretation of complex cases should be clearly separated from samples for confirmatory testing. No samples from screening programmes of asymptomatic people should be selected for confirmatory testing.

### ***4.2 Highlights from rash and fever surveillance programme***

Dr Galina Lipskaya showed the results of the large-scale rash/fever diseases investigation carried out in the Russian Federation as enhanced (active) surveillance (ES) for measles. The patients identified as measles cases by ES were initially diagnosed within a wide range of other diseases. It is strongly recommended to conduct ES and investigation of the suspected measles cases evenly throughout the year. Based on the current age distribution of measles cases in the Russian Federation, samples predominantly from adults should be investigated and it is important to select patients for investigation with fever above 38.0°C in line with the standard measles case definition. Currently ES covers the whole territory of the Russian Federation with a 15% increase in the number of measles cases detected by ES.

The importance of investigating rash/fever cases during years with low measles incidence was highlighted in Dr Lipskaya's presentation. It was shown by additional analysis that not all IgM-positive cases detected by ES were measles cases, and should be discarded. Between 2007 and 2012 more than 18 000 rash/fever cases were investigated; of these 308 were IgM positive and only 173 were confirmed as measles cases. 235 cases were discarded based on investigation of paired sera for measles IgM and IgG, to exclude false positive ELISA results. The time points of the last vaccination should be taken into account for results interpretation, as well as the special decisions of the Classification Commission. Interestingly none of the 173 confirmed measles cases generated a secondary case and none of their contacts developed measles within 21 days after separation from the case. Clinicians involved in ES sometimes identified additional measles cases even in an outbreak context, but in settings with high disease incidence enhanced surveillance may be of a comparatively low benefit as the few additional cases do not really play such an important role. In 2007–2012 with a low measles incidence rate in the Russian Federation, different sampling of rash/fever cases for 100 000 population was tested. Statistical analysis of the data obtained did not confirm advantages of sampling 2:100000 against 1:100000 for detecting additional measles cases. In conclusion, Dr Lipskaya emphasized the importance of both routine and enhanced surveillance for measles at the measles elimination stage.

#### ***4.3 Other viral aetiologies in measles/rubella suspected cases***

Dr Judith Hübschen presented the results of a recently completed study that investigated samples from measles- or rubella- suspected patients reported in Belarus between 2009 and 2011. As Belarus has achieved very good control of measles and rubella, more and more suspected cases are negative for both pathogens and it was interesting for the local laboratory staff and also the physicians to determine which other viruses may be involved in the observed rash/fever disease. More than 850 sera collected during three consecutive years were screened for specific IgM antibodies against measles virus, rubella virus and Human Parvovirus B19 (B19V). The samples that were still negative were further investigated for antibodies against adeno- and enteroviruses and sera from about 150 children up to three years of age were investigated for antibodies against Human Herpesvirus type 6 (HHV6). It was found that more than half of the samples were positive for antibodies against any of the six viruses, with B19V being the most important pathogen overall and in all years. Less than 9% of the samples were positive for either measles or rubella antibodies. Nearly 90% of the measles positives and all rubella positives were at least 15 years old. B19V dominated in basically all age groups. Among the children under 3 years of age, HHV6 was responsible for nearly half of the antibody positives. During the subsequent discussion the reliability of the IgM results for B19V was questioned as there may be false positives or persisting antibodies may be detected. The cost–benefit of screening samples from measles- or rubella-suspected cases for four additional viruses was discussed.

#### ***4.4 Additional laboratory methods – experience of the Regional Office for the Americas***

Dr Joe Icenogle related the Pan American Region's experience with additional laboratory testing for rubella. He stated that secondary vaccine failures are very rare for rubella. In elimination settings the aim is rather to NOT confirm cases and it is necessary to consider all available data including also clinical and epidemiological information. In the United States they use four different case classifications for rubella related to their importation status including “imported virus”, which means that the genotype identified did not occur in the United States but was definitely imported from abroad. Between 2004 and 2011 more than 80% of the rubella cases were isolated, single cases. The four CRS cases notified in that time period comprised one case



of a mother vaccinated a single time with the measles/mumps/rubella (MMR) vaccine and with an unknown source of infection. In 2012 another three CRS cases were confirmed in the United States. On one hand additional information such as data about recent vaccination and travel history are very important, but at the same time it is very difficult with all the information that should be considered to develop a simple protocol or algorithm to be followed. And this is even more complicated as all data are not always available for consideration. At CDC, IgG testing and IgG avidity testing is considered useful for case classification. A patient that is IgM negative but IgG positive is not likely to be a rubella case. If cases of reinfection occur, they normally do not cause any secondary cases and are therefore not likely to be an issue for surveillance. Concerning the CRS case definition, Dr Icenogle stressed that single birth defects are not uncommon and may be due to genetic disorders, but that the combination of defects mentioned in the WHO case definition is quite specific for CRS. In the subsequent discussion, it was mentioned that countries that do not have baseline data about rubella genotypes may not use the classification “imported virus”. The CRS case in the mother was further discussed and it was specified that the follow-up was not ideal as very little information was available, the case was notified very late and the collected specimens had been thrown away and were not available to CDC. The question was raised of how CDC confirms cases of secondary immune response for rubella and it was clarified that normally these people are not ill and therefore are not suspected cases and no samples are collected for laboratory investigation.

Dr Paul Rota said that it is not certain that absolutely all cases of measles are detected and reported in the United States, but he is confident that the system does not miss any outbreaks. Molecular testing is done at state labs or at CDC and the IgM results are not always confirmed. If the samples are collected during the first week after onset of rash, there is a high chance of detecting viral RNA by PCR. In 2013 either IgM testing, PCR or both methods were used in the United States for case confirmation. Dr Rota also shortly described a small outbreak initiated by a 22-year-old woman vaccinated twice in her childhood. Some of the secondary cases were also fully vaccinated. Besides the common laboratory tests such as IgM, IgG and IgG avidity testing, CDC also checked the plaque reduction neutralization (PRN) titers, calculated an IgM/IgG index ratio for the serum collected at the latest time point (a ratio less than 1 suggests a secondary immune response, while ratios above 1 suggest a primary response) and performed molecular testing. CDC considers a very high PRN titer as a suitable biomarker for cases of secondary immune response. A fourfold rise in IgG antibody titers is not always observed in patients with already initially high IgG titers, but a very high PRN titer ( $\geq 40,000$  mIU/mL) clearly points to a secondary immune response. Due to the large ongoing measles outbreak in the Philippines, frequent importations of B3 “Harare” to the United States have been observed. While the outbreak cases genotyped so far are due to B3, it is not clear whether the circulation of D9 has been completely interrupted in the Philippines. In the subsequent discussion the question was raised of whether CDC also looked at differences in viral load between primary infections as compared to secondary immune response cases. At PHE a low viral load was observed in OF samples of secondary immune response cases.

## **Session 5: Molecular external quality assessment**

Chair: Dr Judith Hübschen

### ***5.1 Update on first global proficiency testing panel***

Dr Paul Rota presented an update on the current status of the molecular EQA panel. The distribution of the initial test panel is a bit behind schedule, but panels should have arrived by

mid-March 2014 at the labs participating in the first test round. Over the past years more than 100 test panels were distributed and the experience derived from this gives CDC a good overview of what to expect and how things work. Some of the problems identified were that the Basic Local Alignment Search Tool (BLAST) was used for genotype determination, which probably yields the correct genotype but will not detect small sequencing errors; and cross-contamination was occasionally observed, which is why lower RNA concentrations are now used. CDC is currently monitoring the RNA stability at low concentrations, but has nevertheless already sent out the panels. Initial results show that the low RNA concentrations are stable for at least three months, but the panel seems to be sensitive to humidity. The samples should therefore be tested soon after the seal is broken. The shipment also contains instructions on how to work with the panel. A score sheet is currently being developed and the submission of detected sequences to a special section of MeaNS and RubeNS is planned for the second test round next year. There are currently three scoring criteria (detection of measles or rubella, generation of the amplicons for sequencing and sequencing and genotyping), which will be refined based on the initial results.

## ***5.2 Experience of Instand panel development***

Dr Sabine Santibanez shared experience with the production of a molecular measles panel for the commercial company Instand. The RRL Berlin was mainly involved in the scientific background, the protocol development and the provision of measles strains. The long-term vision of Instand is to develop an MMR panel; starting with measles. The challenges are to produce a large number of identical panels with samples that are stable outside of the freezer and to store and ship the samples at low cost. The initial panel consists of FTA cards loaded with culture supernatant; and for the first test a 10-times dilution series was prepared. After storage of the cards for 2 weeks, one dilution less was detected compared to direct RNA extraction. However, the panel was stable for at least three months when kept at 4°C. The stability testing is ongoing, also at room temperature, and the use of different genotypes is also being considered. Detailed instructions on how to work with the panel are available.

In the subsequent discussion, concerns about not using gloves on the pictures on the instruction sheet and about cross-contamination when punching the disks were raised. Experiments performed at the RRL Berlin did not show any cross-contamination due to the punching of the disks. It was suggested that the head of Instand could be invited to the next Global WHO Labnet meeting to present their serology and molecular panels and also to discuss possibilities to have the WHO molecular panel produced by that company. Dr Brown mentioned that at least one other commercial company, QCMD (<http://www.qcmd.org/>), is planning to produce molecular panels for measles, and that NIBSC now offers the run control that was produced as part of the measles molecular panel produced for ECDC several years ago (using heat-inactivated material provided by PHE; ([http://www.nibsc.org/products/biological\\_reference\\_materials/product\\_catalogue/detail\\_page.aspx?catid=13/168-001](http://www.nibsc.org/products/biological_reference_materials/product_catalogue/detail_page.aspx?catid=13/168-001))). A measles molecular panel comprising 12 samples, different genotypes and different concentrations is already available. CDC would be glad to have a company produce the panel, but the quality needs to be assured.

## **Session 6: Molecular epidemiology of measles and rubella viruses**

Chair: Dr Kevin Brown

## ***6.1 Molecular epidemiology updates***

### **6.1.1 Global specialized laboratory, England**

Dr Kevin Brown explained that the United Kingdom recently had several importations of measles from the Philippines, and presented an example of exposure within an aircraft. The connection between the patients was identified due to an unusual B3 sequence found among several patients. Risk assessment guidelines for infectious diseases transmitted on aircraft (RAGIDA) guidelines recommend that if a patient with measles travelled in an aircraft, all passengers should be contacted and followed up. Guidelines in the United States refer to just the three rows in front and behind of the seat in which the measles patient was sitting during the flight. The data presented showed that this may not be sufficient for measles. According to Dr Icenogle, it may be sufficient for rubella.

### ***6.1.2 Russian Federation and newly independent states***

Dr Sergey Shulga presented a comprehensive overview of measles molecular epidemiology data for the Russian Federation and the newly independent states (NIS). He reported that several different genotypes were detected in the Russian Federation in recent years. The same regions that had a high measles incidence in 2012 also had many cases in 2013, but caused by a different genotype (D4 in 2012 and D8 in 2013). In the Ukraine an outbreak of D4-Manchester is still ongoing, but in the eastern part of the country two different lineages of D8 were found in 2013. An outbreak in Azerbaijan in 2013 was also caused by D4-Manchester and some of these viruses were imported to the Russian Federation. Between 2010 and 2013 at least four different clusters of D8 were observed involving strains found in the Belarus, Russian Federation and Ukraine. Sometimes viruses were imported presumably from Georgia, India and Thailand. Genotype B3 was found in 2012 and 2013. In some regions consecutive outbreaks caused by viruses belonging to different genotypes were detected. While the circulation of D4-Bandarabas seems to be interrupted, D4-Manchester is still found in the region. There were multiple importations of different viruses, especially of genotypes D8 and recently B3 into the Russian Federation. Gaps in measles notifications and susceptible populations were identified. Dr Brown encouraged people to suggest variant names and recommended that if possible the earliest sequence should be used and it should also be submitted to GenBank so that people who are not using MeaNS can refer to and use the sequence. For several of the small outbreaks no epidemiological data to confirm virus importation were available. With the many cases currently detected in the Russian Federation, the country does not seem to be close to measles elimination.

## ***6.2 Challenges in current sequencing strategies***

Dr Paul Rota shared his experience with P and H gene sequence investigation. While the P gene did not help to discriminate between B3 sequences from the United States and Ecuador, the H gene showed a single nucleotide difference. CDC will do complete genome sequencing based on NGS technology to see whether this information will help to discriminate between the strains. For rubella the current sequencing window is perfectly sufficient and more information may only be needed in the future.

Dr Annette Mankertz added that investigation of D4-Hamburg strains showed very few mutations in the H gene and the data did not help to come to any meaningful discrimination between different transmission chains. She emphasized that she does not see a need for additional sequence data at this time.

Dr Mick Mulders showed a few slides about the spread of D4-Enfield. The virus was stable for several years and showed only few mutations after many years of circulation.

Data from the RRL Luxembourg presented at the last Global WHO Labnet meeting also confirmed the low mutation rate and the limited usefulness of P and H gene sequencing to differentiate between different chains of transmission.

### ***6.3 MeaNS – RubeNS update***

Dr Kevin Brown provided a summary of the discussions and main decisions taken during the MeaNS and RubeNS steering committee meetings (cf separate minutes) and recent molecular data from the databases. He compared current possibilities and tools available for the two databases and gave a short overview of current RubeNS developments and submissions to MeaNS during 2013. Participants were strongly encouraged to suggest variant names to MeaNS, using if possible the earliest sequence and also to submit this to GenBank.

### ***6.4 Extended sequencing***

Dr Kevin Brown presented data about the genetic variation in different regions of the measles virus genome. From a cost–benefit point of view, he does not see a current need for additional sequence information, but PHE was nevertheless working on the development of an NGS approach to investigate some strains of interest. After initial limited success, PHE is now using a strategy with 20 overlapping amplicons and are able to get sequence data directly from clinical material (mainly OF samples). PHE has had problems getting a good coverage of the intergenic region. A comparison of sequence data obtained by conventional Sanger sequencing and NGS did not show any differences. The RRL Luxembourg strategy is very similar to what is being developed at PHE with 21 amplicons. Luxembourg was also able to get sequence information directly from clinical material, but also has a problem with the intergenic region. The rapid developments in NGS technology were discussed; complete genome investigations may become easier and more successful in the future. For rubella virus with its high GC content and the low copy numbers in clinical material, an NGS approach may be even more challenging, but it is worth investigating the possibilities.

The meeting concluded with agreement on meeting recommendations.

## **Conclusions and recommendations**

Progress, challenges and lessons learnt were reviewed during the meeting, and solutions and future plans were discussed, leading to meeting recommendations as summarized below:

### **Recommendation 1: Strengthen case-based reporting as a key contribution to verifying measles and rubella elimination**

Case-based surveillance with laboratory confirmation is critical to monitoring the progress of the measles/rubella elimination programme. Appropriate linkage of epidemiological and laboratory data is key to interpret outbreaks quickly, assess country status (verification) and inform decision-making. WHO/Europe therefore developed a specimen-based tool to collect laboratory investigation data.

- In consultation with RRLs, WHO/Europe should upgrade MRLDMS with additional functionalities to allow more flexibility and increased uptake by the Labnet.

- Network laboratories are encouraged to implement the MRLDMS tool.
- Countries should advocate for unique identifier at national level, to be linked with MeaNS as well.
- WHO/Europe and ECDC should continue to coordinate the reporting via Tessy/CISID.

**Recommendation 2: Follow up on accreditation issues and provide tailored technical advice to national reference laboratories to comply with WHO Labnet standards**

Labnet is strong and proficient, has good expertise, and is able to deal with the current workload. Additional capacity may be needed to ensure continued ability to deal with increasing workload, including enhanced molecular surveillance.

- Laboratories submitting samples for confirmatory testing should provide complete laboratory investigation data, origin of samples as well as final result conclusion.
- However, laboratories should only submit samples from suspect cases (measles or rubella case definition) and not screening samples (accreditation checklist to be clarified). Referral testing of complex cases for expert advice should be done separately.
- All laboratories should participate in the assessment for full accreditation. Non-conformity may result in provisional accreditation.
- NRLs should resume samples shipment to a RRL, particularly from NIS to Moscow RRL. Use of FTA<sup>®</sup> and filter papers should be encouraged to overcome cold chain and customs issues.
- National and regional reference laboratories should strengthen bilateral communication.

**Recommendation 3: Enhance molecular surveillance of measles and rubella viral sequences and scale up reporting to MeaNS and RubeNS databases**

Labnet is a main contributor of measles genotyping data and has provided major insights into measles molecular epidemiology worldwide. However, this contribution varies greatly among the subregions. In addition, rubella genetic information is repeatedly missing. Increased efforts are needed to improve the knowledge of molecular epidemiology both for measles and rubella.

- Laboratories are encouraged to consider using alternative sample transportation as this has proved to be a successful tool for genotype surveillance in Labnet.
- Laboratories are requested to provide timely sequence data to the programme through the WHO genetic surveillance databases MeaNS and RubeNS.
- GSL and WHO/Europe should provide additional training opportunities to laboratories not providing sequence data (webinars, regional meetings).
- Labnet needs to advocate at all level to increase molecular surveillance and reporting and provide feedback information to national laboratories on newly submitted sequences.
- Labnet should renew the programmatic and laboratory emphasis on rubella genotyping.

**Recommendation 4: Develop technical guidance for the next steps of measles and rubella elimination in WHO European Region**

Currently there is no need to change the laboratory techniques and investigation strategy of measles and rubella suspected cases. However, it is likely that additional laboratory confirmatory methods (such as IgG, IgG avidity, PCR, investigation of a second serum) will be necessary for suspect case classification when approaching and maintaining measles and rubella elimination because of decreased positive predictive value of IgM serology. The Region should anticipate and address this challenge now.

- WHO should develop guidance for case classification of measles and rubella in a low incidence setting (using additional confirmatory methods).
- WHO needs to provide global serosurvey guidelines as a tool to assess population immunity against measles/rubella in Member States.
- Labnet should provide laboratory investigation results on the role of measles transmission from vaccines to contacts (reinfection, secondary vaccine failure). Meeting participants are requested to provide additional data on this topic.
- Countries are requested to continue investing in rash and fever surveillance as this may offer a tool to assess the performance of surveillance in countries approaching elimination.

## The WHO Regional Office for Europe

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

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