

**11TH MEETING OF THE MEASLES/RUBELLA
REGIONAL REFERENCE LABORATORIES
OF THE WHO EUROPEAN REGION**

**14-15 MARCH 2016
Berlin, Germany**



KEYWORDS

ACCREDITATION
COMMUNICABLE DISEASE CONTROL
DISEASE ELIMINATION
SURVEILLANCE
EPIDEMIOLOGY
IMMUNITY
LABORATORIES
VERIFICATION
MEASLES
RUBELLA

Address requests about publications of the WHO Regional Office for Europe to:

Publications

WHO Regional Office for Europe

UN City, Marmorvej 51

DK-2100 Copenhagen Ø, Denmark

Alternatively, complete an online request form for documentation, health information, or for permission to quote or translate, on the Regional Office website (<http://www.euro.who.int/pubrequest>).

© World Health Organization 2016

All rights reserved. The Regional Office for Europe of the World Health Organization welcomes requests for permission to reproduce or translate its publications, in part or in full.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either express or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use. The views expressed by authors, editors, or expert groups do not necessarily represent the decisions or the stated policy of the World Health Organization.

Contents

- Abbreviations 3
- Acknowledgments 5
- Executive summary 5
- Session 1 – Global and regional updates..... 6
- Session 2 – Laboratory contribution to verify measles and rubella elimination..... 13
- Session 3 – Laboratory data management..... 16
- Session 4 – Molecular diagnostics and epidemiology 17
- Session 5 – Serology 23
- Session 6 – Future plans & recommendations..... 26

Abbreviations

ASU	Annual Status Update
AFRO	African Region
B19V	Parvovirus B19
CDC	United States Centers for Disease Control and Prevention
CRI	congenital rubella infection
CRS	congenital rubella syndrome
DRC	Democratic Republic of the Congo
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
ETAGE	European Technical Advisory Group of Experts on Immunization
EU	European Union
EUR	European Region
GMRLN	global measles/rubella laboratories network
GSL	global specialized laboratory
IQC	internal quality control
IgG	immunoglobulin G
IgM	immunoglobulin M
LabNet	laboratory network
mEQA	molecular External Quality Assessment
MCV	measles-containing vaccine
MeaNS	Measles Nucleotide Surveillance Database
MeV	measles virus
MMWR	Morbidity and Mortality Weekly Report (CDC)
MR	measles/rubella
MRLDMS	Measles and Rubella Laboratory Data Management System
MRCV1	first dose MCRV
MCRV2	second dose MCRV
MMR	measles, mumps and rubella
NGS	next generation sequencing
NIS	newly independent states
NL	national laboratory
NRL	national reference laboratory
NVC	national verification committee
OF	oral fluid
PAHO	Pan-American Health Organization
POCT	point of care test
PCR	polymerase chain reaction
PHE	Public Health England
PHL	public health laboratories
POCT	point of care test
PP	proficiency panel
PT	proficiency test
RLC	Regional Laboratory Coordinator
RKI	Robert Koch Institute
RNA	ribonucleic acid
RRL	regional reference laboratory
RubeNS	Rubella Nucleotide Surveillance database

RVC	Regional Verification Commission for Measles and Rubella Elimination
SIA	supplemental immunization activity
SEAR	South East Asian Region
Tessy	The European Surveillance System
USD	United States Dollars
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
VIDRL	Victorian Infectious Diseases Reference Laboratory
VPD-RC	vaccine-preventable disease reference centers
WER	Weekly Epidemiological Report (WHO)
WPR	Western Pacific Region

Acknowledgments

We are indebted to the WHO European Measles and Rubella Regional Reference Laboratory at the Robert Koch Institute, Berlin, Germany for graciously hosting the meeting.

Executive summary

The 11th meeting of the Measles/Rubella Regional Reference Laboratories of the WHO European Region took place in Berlin, Germany on 14 -15 March 2018.

Representatives of the following institutions/laboratories attended the meeting:

- The three European Regional Reference Laboratories: Robert Koch Institute, Berlin, Germany; Luxembourg Institute of Health, Luxembourg City, Luxembourg and G.N. Research Institute of Epidemiology and Microbiology, Moscow; Russian Federation,
- Global Specialised Laboratories: Public Health England, London, United Kingdom; Centers for Disease Control and Prevention (CDC), Atlanta, United States of America,
- Regional Reference Laboratory for Pan America Region Public Health Agency of Canada, Winnipeg, Canada,
- The Regional Verification Commission for the Elimination of Measles and Rubella,
- The European Centre for Disease Prevention and Control (ECDC),
- WHO staff from the Regional Office and Headquarters.

The participants were updated on the progress of the Measles/Rubella programme at the global and regional levels and discussed regional issues and future plans pertaining to accreditation, molecular EQA, enhancing molecular detection/surveillance, verification of elimination, training and publications. Based on the presentations and discussions, the participants agreed on a set of operational recommendations on the different topics addressed during the meeting.

Background

The WHO European Regional Office (EURO) coordinates a laboratory network (LabNet) of specialised centres that conduct diagnosis and surveillance for measles and rubella (MR). The MR LabNet was set up in 2002 and is constituted of 73 laboratories distributed across the region's 53 member states (MS). Three Regional Reference Laboratories (RRLs) in Berlin, Luxembourg and Moscow and a Global Specialised Laboratory (GSL) in London supervise and coordinate National Reference Laboratories (NRLs) and Sub-National Laboratories (SNLs) and conduct assay development and implementation across the region.

Reliable diagnosis and effective surveillance of MR become increasingly critical as the European Region (EUR) pursues the elimination of endemic MR. The region's GSL and RRLs meet on an yearly basis to exchange information, address issues, share achievements and discuss future directions to promote the endemic MR elimination programme in EUR. This report summarises the presentations and discussions that occurred throughout the meeting and closes with the recommendations resulting from these interactions

Pr L Schaade , director of Robert Koch Institute welcomed the participants

Session 1 – Global and regional updates

Chair: Prof. Dr. Annette Mankertz

Regional Measles and Rubella program update

Dr. Patrick O'Connor (WHO Regional Office for Europe)

Despite the large amount of progress made over the last three decades — a 98% reduction in the number of measles cases since 1993 and a 98% reduction in rubella incidence between 2000 and 2011 — the measles and rubella elimination targets set for the European Region were missed for 2015. Dr. O'Connor updated meeting participants on regional epidemiological situation. He also addressed the challenges the Region faces in the coming year, and the efforts required to achieve elimination going forward.

The widespread use of measles-containing vaccines has been crucial to the dramatic decline in the number of measles cases. Regional coverage with the first measles-containing vaccine (MCV1) has been maintained above 95% for several years; nevertheless, a significant number of measles cases continues to occur. In 2015, a total of 7399 cases was reported, with large outbreaks occurring in Germany, Kazakhstan, the Russian Federation, Georgia, France, and Turkey. Much like 2014, the largest proportion of cases has occurred in individuals older than 20 years, an age group more likely to be unvaccinated or to present with unknown vaccination status. However, some countries did show age-specific distributions: Turkey and Kazakhstan showed the highest burden in children under 9 years; Germany and France showed the highest burden in 10-29 year olds; and Georgia and the Russian Federation showed the highest burden in the over 30 age group. These differences further underscore that such a heterogeneous region will face unique, country-specific challenges in their efforts and strategies towards measles and rubella elimination.

Reported measles cases occurred in both the general population and in specific population groups considered at risk, including several outbreaks occurring in Roma and Sinti, Traveller, followers of anthroposophy, and Ultra-orthodox communities/populations. Many outbreaks have had their foci in health care facilities, with unprotected health care workers responsible for disseminating cases. Closing the immunity gap in this group by increasing advocacy for mandatory vaccination is thus of great priority. The WHO is currently developing online training materials to educate health care workers on the importance of vaccination for both their patients and themselves (projected 2016 launch date).

A total of 1709 rubella cases was reported in the Region in 2015. Like 2013, and 2014, Poland continues to have the highest burden (n = 1 508). Georgia follows with 94 cases and then Germany with 49 cases.

Despite not meeting the 2015 measles and rubella elimination goal, many countries in the Region have provided evidence for absence of endemic measles and/or rubella transmission through their commitment to strong immunization and surveillance programs. For those countries still in the process of achieving elimination, it will be crucial for them to enhance high quality surveillance and to increase population immunity by improving routine immunization coverage and/or closing immunization gaps through targeted supplemental immunization activities (SIAs). Increasing the efficiency of the outbreak response — as well as expediting resource mobilization — is of major

importance as well. Embarking on these challenges will require concerted and targeted efforts, including fostering more partnerships, increasing political will, maximizing communication pipelines, and reinforcing a high level of commitment to elimination.

Regional Measles and Rubella LabNet update

Dr. Myriam Ben Mamou (WHO Regional Office for Europe)

Participants were updated on the progress made towards the implementation of the recommendations agreed on during the 10th RRL meeting. Dr. Ben Mamou highlighted the activities and performance of the Regional Measles and Rubella Laboratory Network (MR LabNet) over the last year, with special focus on the challenges and action plans for 2016.

The Regional office has conducted nine on-site accreditation visits to Bosnia and Herzegovina, Italy, Spain, Kazakhstan, Georgia, the Russian Federation, United Kingdom and Luxembourg. Epidemiological assessments have been integrated into many of these laboratory visits in an effort to provide more comprehensive support.

Capacity building activities in the past year have included individual meetings in Spain, Kazakhstan, and Republic of Moldova, as well as molecular workshops and MeaNS trainings in the Russian Federation and newly independent states (NIS). Distributing ELISA kits, FTA[®] cards, CDC molecular reagents, EQA panels, and proficiency tests to the NRLs in the Region was of particular focus. The Regional Office has also managed the overhaul of MRLDMS, now called MRLDMS-2.

In 2015, 68 NRLs and SNLs participated in the accreditation process, with 67 laboratories achieving accreditation and one laboratory achieving provisional accreditation. Overall, EQA performance was excellent, with proficiency testing and IgM accuracy greater than 90% for all participating laboratories. Nevertheless, certain challenges still persist: only 20% of laboratories have timely genotype reporting to WHO databases (MeaNS and RubeNS); only 30% of laboratories have established internal quality control (IQC) procedures; and, only 47% of laboratories report IgM results to the surveillance system within the recommended four day period.

The MR LabNet received 29843 specimens for measles and rubella testing in 2015, of which 20% were positive for measles and 2% were positive for rubella. D8-Rostov on Don and D8-Chui predominated as reported to MeaNS. Less genotype information is available for rubella, however, with only a limited number of sequences reported to RubeNS. Of the sequences reported, genotype 2B predominated (from Germany, Portugal, the Russian Federation, and the UK).

In 2016, the Regional Office is planning to roll out the new MRLDMS-2, contribute to accreditation visits and capacity building activities throughout the entire network, and facilitate communication and information exchange between laboratories. Continuous advocacy for high quality laboratory verification data and rubella genotyping, establishing more epi-lab linkages, and promoting the timely and complete reporting to WHO databases is of foremost priority for the Regional Office going forward.

Update on WHO global program and GMRLN LabNet

Dr. Mick Mulders (WHO Headquarters)

Dr. Mulders provided an update on the efforts underway to eliminate measles and rubella in the six WHO Regions. He also presented the recent developments of the Global Measles and Rubella

Laboratory Network (GMRLN), the challenges the network has faced, and the recommendations and long-term goals that have been made for 2016 and beyond.

Global measles update: Control milestones for measles have been missed and regional measles elimination targets are off-track for 2015. Approximately 2.4 billion USD have been invested in global measles control, yet progress has been slow or stagnant over the last five years with only one region on track for reaching elimination (PAHO). The scorecard against the three global targets reveals that all elimination milestones were not met for 2015: MCV1 coverage remains static — with only 63% of countries achieving greater than 90% coverage — and the rate of decrease in both measles incidence and measles mortality is still progressing too slowly. Furthermore, many measles outbreaks continue to occur throughout the world, with the greatest proportion of cases in children between 1-4 years of age, most of which did not receive MCV1 (or had less than the two recommended doses). The greatest measles burden continues to be seen among the six countries with the weakest health systems, particularly in India and Pakistan; considerable progress can only be made globally if concerted efforts are made to strengthen the systems in these countries necessary to prevent missed immunization opportunities.

Global rubella update: Rubella elimination targets were not met in 2015, with outbreaks still occurring. Rubella reporting and surveillance continues to be weak in many countries and most regions have not yet set elimination goals, except for two (SEAR [2020] and EUR [2015; currently off-track]). A gradual increase in rubella vaccine coverage has been observed, however, with 14 countries introducing the vaccine between 2013 and 2015; nevertheless, over half of the world's children are still not being reached. The greatest reported incidence is generally localized to the countries where rubella vaccination has not yet been introduced — underscoring that the failure to integrate rubella and CRS prevention with measles elimination represents a major missed opportunity for vaccine integration and disease control.

GMRLN update: Recent developments at the GMRLN include implementing a molecular External Quality Assurance (mEQA) program in cooperation with CDC and INSTAND e.V.; standardizing methods for seroprevalence studies; revising the WHO Laboratory Manual; forming a whole genome/next generation sequencing working group; evaluating point of care assays to detect IgM and IgG for measles and rubella; and developing new serologic assays to support seroprevalence studies and case confirmation.

The GMRLN is anticipating workload, resource, and programmatic challenges in the coming years. A number of factors can quickly overwhelm laboratory capacity and staff workloads, with concerns surrounding the efforts needed for verifying elimination through genotyping; carrying out serosurveys in pre-elimination settings; establishing case-based surveillance; and expediting outbreak responses. Competing priorities — i.e., with other rash-causing viruses such as Ebola, MERS-CoV, ZIKV — will also have implications for measles and rubella. The lack of baseline genotype information for rubella, and the failure to report genotype information to sequence databases (RubeNS) by many countries, is also of significant concern from a programmatic perspective.

GSL update: United Kingdom

Dr. Kevin Brown (GSL United Kingdom)

Public Health England (PHE), now a part of the Department of Health, has been undergoing restructuring, personnel changes, and austerity cuts over the last three years, including the formation of a National Infection Service — which has been advantageous for reuniting epidemiology and laboratory services within the same unit — and the formation of a Clinical Service Unit, which will act as a commissioned service for measles and rubella IgM/IgG/total IgG and RT PCR testing. In the last two quarters of 2015, there has been high coverage and uptake of the measles vaccine in the UK. 95% of five-year olds have attained MMR1 coverage and 90% of children in this same age group have been fully vaccinated with both doses. A dramatic fall in the number of measles cases has been observed since the large measles outbreak in 2012/2013 in England and Wales; supplemental immunization campaigns targeted at school age children were responsible for this dramatic decline. Since then, there have been only sporadic measles cases reported in the region, likely the result of importations (B3, D4, D8, and H1 genotypes).

A second round of measles molecular proficiency testing took place in 2015 at the GSL. Proficiency panels were sent to 20 PHE laboratories, including laboratories in Scotland, Wales, and Northern Ireland. The panel included nine measles-positive samples and one measles-negative/cellular control sample. Results thus far have shown that only two laboratories use cellular controls in their routine diagnostics.

In 2015, there were two cases of rubella and two CRS cases. Both CRS cases were in children born to mothers not originally from the UK (yet had been living in the UK since childhood). In both cases, investigations during pregnancy were not appropriate since both mothers experienced rash-illness yet rubella IgG screening was falsely deemed sufficient to exclude the disease.

Due to the combination of inappropriate interpretation of results and the anxiety over false negatives, the number of susceptibles for rubella during pregnancy has been increasing since 2005. An outcome of this large increase in specimens has been the decision to cease the antenatal screening program for CRS as of April 2016. Instead of screening all samples for rubella IgG, the guidelines on rash and pregnancy will be re-written. Women will be educated on reporting rash-illness and non-vaccinated women will be recommended to be vaccinated post-partum by their midwives/health care providers.

The performance of the Microimmune assay continues to be of concern, with increased numbers of equivocal and false positive results being observed since 2012. Assay performance will need to be reoptimized.

Studies on next generation sequencing (NGS) of measles variants have been underway at the GSL in 2015. During the 2012/2013 outbreak, it was found that there was only one nucleotide difference between the Taunton and Swansea D8 genotypes, raising questions of whether genetic drift or multiple importations were implicated during the outbreak. Sequencing analyses of the whole genome (WGS), the non-coding region (M/F NCR), and the N-450 region were compared for 50 specimens from the outbreak to further characterize these specimens. From their evaluations, they concluded that the N-450 sequencing window is still the best approach in low-resource and endemic transmission settings; yet, widening the sequencing window may allow for better characterisation of MeV outbreaks, despite being expensive and time-consuming. In well-resourced countries

approaching elimination, however, the GSL recommends that M/F NCR and/or WGS may be a good complement to N-450 sequencing.

GSL update: United States/Measles

Dr. Paul Rota (GSL United States)

Provisional data from 2015 indicates that there were 189 measles cases spread across 25 states and the District of Columbia. 89% of these cases were either unvaccinated or presented with unknown vaccination status; almost all of these cases (94%) were import-associated.

Five measles outbreaks occurred in 2015, with the largest outbreak (n=147) having its foci at Disneyland in California (genotype B3, Harare strain). The second largest outbreak (n=15) occurred in a day-care center in Colorado (genotype B3). All outbreak-associated specimens were sequenced, with B3, D9 and D8 predominating. As of 2016, two cases of measles have been reported with no large feeder outbreaks identified.

The CDC, as well as four vaccine-preventable disease reference centers (VPD-RC), use RT-qPCR for measles case confirmation. This method is useful when samples are collected too early for IgM to be detectable, or when serum was not collected at all. Moreover, it is an especially valuable method when testing reinfection cases — i.e., when the IgM response is often delayed or absent. In 2015, close to 1000 samples were tested by the CDC and the VPD-RCs, of which 243 sequences were generated. 79 of these sequences — a higher number than previous years — were identified as being vaccine-strain A. In these cases, a patient had developed a vaccine-induced rash within 21 days of measles vaccination, mimicking a true wild-type infection. Because it is not possible to distinguish between vaccine and wild-type strains based on their Ct values using their current diagnostic RT-qPCR (i.e., all must be genotyped) the CDC will be implementing a vaccine-specific RT-qPCR developed in 2015 by Dr. Severini and colleagues at the Public Health Agency Canada. This method will greatly increase the turnaround time required for identifying such cases.

Specific activities of the US CDC in 2015 have included: the distribution of EQA proficiency panels and FTA® cards; training visits in AFRO and WPR as well as in-house training sessions; the formation of a next generation sequencing (NGS) working group; the development of an MBA assay and the Luminex® platform; and the research on microneedle vaccine.

GSL update: United States/Rubella

Dr. Joseph Icenogle (GSL United States)

The CDC International Measles and Rubella Laboratory Capacity Review Tool has been developed and is in the process of being rolled out. The tool is designed to assess the capabilities and capacities of laboratories that carry out measles and rubella diagnostics. Not only can laboratories determine their strengths and challenges, but they can also determine if they have enough capacity for CRS and rubella surveillance. The tool is currently being tested at an NRL in Ghana and modifications will be made based on their evaluations and feedback.

The US CDC is focusing efforts on researching the pathogenesis of CRS in fatal cases since lung biopsies from these patients have been found to be infiltrated with macrophages containing rubella virus. Further efforts are also underway to validate and extend upon the results from a study in 2014 suggesting that rubella virus is the likely cause of granulomas in children with primary immunodeficiency. The persistence of rubella virus in Fuchs Uveitis and CRS disease states, in addition

to carrying out transcriptome analysis of rubella-infected human umbilical vein endothelial cells (HUVECs), is also under examination.

A paper on the molecular epidemiology of rubella virus in the Democratic Republic of the Congo (DRC) is in press at the Journal of Medical Virology. Another paper is under final review at Euro Surveillance focusing on rubella molecular surveillance in Romania. In both studies, sera were used as samples for genotype determinations.

RRL Berlin Update

Prof. Dr. Annette Mankertz (RRL Berlin)

Prof. Dr. Mankertz informed participants on the measles outbreak that affected 1358 people in Berlin, Germany between October 2014 and August 2015. She also provided updates on the specific activities of the RRL Berlin over the past year.

The Berlin outbreak had its foci in a temporary shelter for people seeking asylum, with the index case originating in a family who traveled from Bosnia and Herzegovina. At the beginning of the outbreak, more than half of the cases were reported in individuals living in these shelters, with children between one and five years of age most affected. However, as the outbreak dragged on into 2015, almost 90% of the cases were reported among the general population, a large fraction among adults. Importantly, despite being initiated within the refugee population, the outbreak was largely fueled by the high number of unprotected residents in the general Berlin population. Primary vaccine failure was not implicated in the outbreak and secondary vaccine failure was of minimal epidemiological relevance.

The outbreak resulted in one fatal case. The boy, born in July 2013, was attending a day-care facility and had suffered from a variety of infections, including lab-confirmed Coxsackie and Parvovirus B19. Because of his 'delicate' constitution, the pediatrician decided that the MMR-vaccine should be postponed. In February 2015, the child developed a serious upper respiratory infection and was transferred to a hospital in Berlin where his condition deteriorated rapidly. While there, he developed exanthema and it was confirmed that he had a measles virus infection. He passed away shortly thereafter and an autopsy determined that his heart was de-rounded, thickened, and almost doubled in size — a sign of inflammatory cardiomyopathy most likely induced by the Coxsackie virus infection he had contracted previously. It was emphasized that this child would have clearly benefited from MMR-vaccination, but vaccination skeptics had assumed he was too ill, including his pediatrician.

Endemic circulation of genotype D8 was reestablished in Germany during the outbreak, with the transmission chain lasting longer than 13 months. Rostov on Don was identified as the major named-strain, accounting for 341 of the cases; however, a large number of D8-variants were also identified. The quality of surveillance was generally high in 2015 with almost all federal states submitting sufficient samples. Nevertheless, the eastern federal states still have lower reporting rates than the western federal states — a historical trend observed since German Reunification. In cooperation with NAVKO, the National Verification Commission, a report will be published on the genotyping results from this outbreak.

Meeting participants were updated on the specific EQA measures carried out in 2015 by the RRL Berlin. In May of 2015, the RRL was re-accredited by DAkkS (the German Accreditation body) with zero deviations; the RT-qPCR used in measles diagnostics was also accredited. The RRL is currently in the process of evaluating results from serology PT and confirmatory testing from the 18 countries under its supervision. Furthermore, a National Ring Trial had been established in cooperation with INSTAND e.V. for the detection of measles, mumps and rubella.

Four papers on topics related to measles transmission, outbreaks and molecular epidemiology have been submitted/published. With regard to rubella, three papers have been published on topics related detection and quantification, gene expression profiling, and apoptotic signaling.

RRL Luxembourg update

Dr. Judith Hübschen (RRL Luxembourg)

The NRL Luxembourg received nine specimens from six patients for measles and/or rubella testing. Only two of the patients fulfilled the WHO criteria for measles suspected cases, and both were negative.

The RRL Luxembourg distributed serum proficiency panels, molecular panels, filter papers, microelute cards and CDC practice kits to the NRLs under its supervision. 16 of the 17 NRLs with results available at the time of the meeting achieved 100% concordance in serum proficiency panel testing for measles. Furthermore, 14 of 17 NRLs achieved 100% concordance in rubella proficiency panel testing. Since 2015, more alternative specimens such as dried serum and blood spots and oral fluid than liquid serum are sent for confirmatory testing.

The RRL provided support with measles IgM testing for Médecins Sans Frontières (Doctors without Borders) in the Democratic Republic of Congo (DRC) when the DRC NRL was running short of reagents that were blocked for weeks at customs. They also assisted the NRL in Serbia with IgG avidity testing and sequencing/genotyping. NRLs in Republika Srpska, Federation of Bosnia and Herzegovina (BIH), and Georgia also sent specimens to the RRL Luxembourg for RT-PCR and/or sequencing and genotyping. Urine samples of confirmed measles patients were submitted by the NRL in Israel for characterization of additional regions of the measles virus genome in an attempt to differentiate between transmission chains; experiments are currently ongoing.

Clinical samples from two different rubella patients were received from the NRL in Portugal for genotyping and virus isolation. One sample from a CRS case was positive and the virus was characterized as belonging to genotype 2B.

Prof. Dr. Claude P. Muller joined WHO officials for onsite accreditation visits to the two NRLs in BIH and to the NRL in Georgia. Collaborative outbreak reports were published with the NRLs in Serbia and BIH.

RRL Moscow update

Dr. Sergey Shulga (RRL Moscow)

The RRL in Moscow supervises a network consisting of 10 national (NRL) and 13 subnational laboratories (SNL) within the Russian Federation and newly independent states (NIS). Dr. Shulga updated meeting participants on the activities within the network and also provided molecular surveillance data from 2015.

17 laboratories participated in proficiency testing in 2015; 12 passed with perfect scores for both measles and rubella and five laboratories were still pending at the time of this meeting. The RRL Moscow received 1585 samples for confirmatory testing from 22 laboratories within their network. All 22 laboratories passed with 100% concordance for both rubella and measles. Additionally, three laboratories participated and passed proficiency testing from the INSTAND e.V. cooperation.

Kyrgyzstan reported 21343 cases of measles in 2015, followed by Kazakhstan (n = 2341) and Russian Federation (n = 843). Genotyping data reveals that there was a predominance of different D8 lineages with origins outside of the NIS region. Prolonged simultaneous transmission of different measles virus lineages — sustained by multiple repeated importations of infection — has thus been implicated. Local transmission was mostly involved within pockets of susceptibles, i.e., in unvaccinated adults and young infants, in the Roma population, and among certain religious groups. At the end of 2015, there was a decrease in measles incidence, possibly due to interruption of local transmission.

Ukraine reported 241 cases of rubella in 2015, followed by Kyrgyzstan (n=101) and Russian Federation (n=25). Rubella genotyping data demonstrates probable links between local cases and imported ones. There was one CRS case reported by the SNL in Rostov-on-Don.

Grant activities at the RRL Moscow have included the procurement of laboratory equipment, reagents, and supplies for the NRLs and SRLs under its supervision; joint meetings between epidemiologists, clinicians, and laboratories — as well as on-site trainings and workshops — were also convened over the last year. ELISA control serum samples and panels were developed, validated and implemented as reported by Dr. Mamaeva.

Session 2 – Laboratory contribution to verify measles and rubella elimination

Chair: Dr. Sergey Shulga

Best practices and lessons learned from laboratory information in 2014 NVC Annual Status Updates

Dr. Myriam Ben Mamou (WHO Regional Office for Europe)

Since 2012, national verification committees (NVC) now established in 50 out of 53 Member States, have been submitting annual status update (ASU) reports to the European Regional Verification Commission for Measles and Rubella Elimination (RVC). The reports are evaluated by the RVC to determine the elimination status of measles and rubella — defined as either interrupted transmission for 36 months (or more) or no endemic transmission — and to provide country-specific recommendations based on their assessment of the following: disease epidemiology of measles, rubella and CRS; surveillance performance; population immunity; sustainability of the national immunization program; as well as any supplemental information/activities. In March 2015, the 2014 ASU report was revised to include three additional sections:

- i) The source of laboratory confirmation for each case of measles, rubella or CRS. Sources may be *WHO-accredited Laboratories* (i.e., NRLs/RRLs), *Proficient Laboratories* (i.e., non-WHO/accredited laboratories using validated assays and passing reputable EQA programs), *Other Laboratories* (i.e., laboratories with no information available on proficiency, EQA, or accreditation status), and/or *Unknown*.
- ii) The standard laboratory confirmation strategy employed as the first line of investigation, delineated as either: *IgM serology*, *Molecular detection*, or *Other*.

- iii) The relevant molecular epidemiology information from each outbreak as well as a separate section for sporadic cases. This is to monitor chains of virus transmission through genetic sequencing and to distinguish endemic cases from imported and import-related cases.

Based on these updates to the form, it was revealed that 30 of 47 Member States have confirmed 88-100% of their cases using WHO-accredited or proficient laboratories with only three Member States not utilizing either. With regard to their confirmation strategies, 32 Member States use IgM serology as the first line of investigation, 11 use “other” methods, and four use molecular detection techniques. More information on genotyping and better linkage of chains of transmission for measles was an improvement seen with the updated form; nevertheless, only 65% of the Member States that submitted molecular epidemiology information could genotype more than 80% of infection chains, and of the 22 Member States who reported rubella cases, only two provided genotype information.

Achieving high quality verification data from all Member States is of foremost importance, especially increasing genotyping of infection chains and improving the sensitivity and quality of the rubella and CRS surveillance systems. Increased engagement and advocacy of the MR LabNet in the verification process (during accreditation visits, in particular) and facilitating more collaboration and information exchange between epi-and-lab is necessitated going forward. Furthermore, increasing the number of accredited WHO-reference laboratories, as well as monitoring the proficiency of non-WHO labs, by, for example, confirming the sensitivity of non-IgM confirmation strategies via mPT cross checks, is highly recommended.

Highlights from the last RVC meeting (Oct 2015) and needs for 2016 process

Dr. Günter Pfaff (Regional Verification Commission)

The European Regional Verification Commission for Measles and Rubella Elimination (RVC) convened for the fourth time on the 26th-29th October 2015 in Copenhagen, Denmark. The committee assessed the status of measles and rubella elimination in the WHO European Region for the period 2012-2014. Their assessments were based on the 50 country reports submitted for that period by national verification committees (NVCs) established in these countries.

The RVC takes into consideration a variety of factors when determining a Member State’s elimination status, including disease and molecular epidemiology; surveillance performance; population immunity; sustainability of the national immunization programs, as well as any supplementary immunization activities underway.

In order to demonstrate elimination, a country has to sufficiently show that i) endemic measles and rubella cases have not occurred for ≥ 36 months, ii) the disease surveillance system is amply sensitive, specific, timely and complete to detect cases if they occurred, and iii) the absence of endemic cases is supported by genotyping evidence. If data are missing or are inconclusive, then in the interest of the verification process, these countries are still considered to have endemic transmission by the RVC – this is in contrast to previous years, when evidence of the measles or rubella elimination status in these countries was labeled as “inconclusive”.

Based on their assessments, the RVC determined that 21 Member States (40%) provided sufficient evidence over the last three years demonstrating the elimination of endemic measles transmission. Furthermore, 20 (38%) of the Member States have demonstrated the elimination of endemic rubella transmission. Not quite reaching elimination — but showing interrupted transmission for a period of

12 to 24 months — was demonstrated by 11 (21%) and 12 (23%) of the Member States for measles and rubella, respectively. 18 Member States continue to have endemic transmission of measles and 18 Member States continue to have endemic transmission of rubella.

The RVC has concluded that little progress has been made on the status of regional measles and rubella elimination between 2012 and 2014. Links between epidemiologic and laboratory surveillance have not been formalized or established in many countries, and surveillance sensitivity remains a major concern. Genotyping information is of increasing importance in the investigation of infection chains, both national and cross-border, yet many countries do not report or measure such data. Despite improvement in the timeliness, quality, and completeness of the country reports, the RVC is concerned that several documents are missing from a few countries with functioning NVCs, that several reports contained incomplete or inconsistent information, and that the verification process in Albania, Monaco and San Marino has not yet been initiated. Without complete documentation from all 53 MS, regional verification will not be possible.

The complete RVC report can be found here: <http://www.euro.who.int/en/health-topics/disease-prevention/vaccines-and-immunization/publications/2016/4th-meeting-of-the-european-regional-verification-commission-for-measles-and-rubella-elimination-rvc-2016>

Verification Quality Virologic Surveillance

Dr. Paul Rota (GSL United States)

Dr. Rota updated meeting participants on the recommendations for obtaining high quality surveillance data outlined in the weekly epidemiologic report (WER) published in July 2015. He also provided a review of the measles genotypes currently circulating globally.

High quality virological surveillance — achieved through the consistent and timely reporting of sequence data from outbreaks and sporadic cases to the measles nucleotide surveillance database (MeaNS) — is fundamental to the verification of measles elimination. The updated recommendations for MeaNS users outlined in the WER are as follows:

- i) All wild-type sequences should be submitted to the database and not just those deemed representative;
- ii) Sequences should be submitted within 2 months;
- iii) Currently circulating strains should be linked to named-strains based on the N-450 sequencing window to help identify transmission chains and potential sources of importation;
- iv) Genotype information should be obtained from at least 80% of all chains of transmission, including sporadic and two-case chains;
- v) Laboratories should work with surveillance staff to encourage collection of samples for viral detection and genotyping. RVCs and NVCs should reinforce the need for complete viral surveillance during the verification process;
- vi) Vaccine-derived sequences should be omitted from the database

Measles virus diversity is decreasing globally. Of the 11 wild-type genotypes detected since 2005, six are still currently circulating: B3, D4, D8, D9, G3 and H1. As of July 2015, there were 5 named-strains for genotype B3; 11 for genotype D4; 8 for genotype D8; 2 for genotype D9; and 6 for genotype H1. In the United States in 2015, B3 (Harare & Allada), H1 (Hong Kong), D8 (Rostov on Don & London), and D4 (Ontario and Brussels) predominated.

The WER can be found here: <http://www.who.int/wer/2015/wer9030.pdf?ua=1>

Session 3 – Laboratory data management

Chair: Dr. Kevin Brown (GSL United Kingdom)

MRLDMS-2 update

Dr. Myriam Ben Mamou and Mr. Simarjit Singh (WHO Regional Office for Europe)

Dr. Ben Mamou and Mr. Singh, in collaboration with others at the WHO, are in the final stages of development of the new Measles and Rubella Laboratory Data Management System (MRLDMS), henceforth called MRLDMS-2. The new system has a tentative release date of May 15, 2016 and training sessions for the NRLs will take place in the last week of June 2016 in both English and Russian at the annual LabNet meeting to be held in Montenegro.

A major benefit of the new system is that data can be imported and uploaded in bulk, thus reducing the burden of entering each specimen by hand. Specimen-based details remain mostly the same, yet there has been an added field for entering the final result classification required for accreditation purposes. Laboratories can also insert their own custom fields, with as many parameters as they wish, according to their profile and their needs and reports can also be made directly within the program and exported to Microsoft Excel.

Another major improvement is that specimens can be linked to epi identifiers, i.e., if a laboratory knows that a specimen is linked to outbreak, they can create a unique outbreak identifier mapping all associated specimens together.

Update from ECDC — Measles and Rubella surveillance

Dr. Sabrina Bacci (European Centre for Disease Prevention and Control)

The European Centre for Disease Prevention and Control (ECDC) was founded in 2004 with the aim of providing infectious disease surveillance, training, epidemic intelligence, outbreak support, evidence-based communication and technical reports to EU/EEA member states. Furthermore, in their efforts to contribute to measles and rubella elimination targets, their multidisciplinary team also provides expertise and support on vaccination programs to identify underserved groups and increase vaccine coverage throughout the region.

The ECDC issues biannual surveillance reports on epidemiology and the geographical spread of measles and rubella from case-based data collected within the The European Surveillance System (TESSy). Additionally, the ECDC produces user-friendly and publically accessible Surveillance Atlases on a monthly basis for measles and rubella (as well as 29 other infectious diseases). This web-based tool allows users to easily visualize maps, diagrams, time series, distributions, and tables based on defined indicators. Surveillance Atlases for measles and rubella, among other diseases, can be found here: <http://ecdc.europa.eu/en/data-tools/atlas/Pages/atlas.aspx>

Measles Surveillance: There has been a large reduction in the number of measles cases in the EU/EEA region over the last five years, yet certain countries continue to report cases. Of the 30 countries that report case-based data in 2015, 3969 cases of measles were reported in total with Germany accounting for 62% of the cases and the only measles-related death. Measles incidence was below one case per million inhabitants in 13 countries. Eight countries reported zero cases. Most cases (85%) were reported in unvaccinated people.

During the period 2011-2016 (until January), 70% of laboratory confirmed measles cases were confirmed by IgM. In the same time frame, over 7000 cases have been outbreak-associated, with only 67% of cases possessing an outbreak identification code. A total of 1521 cases during the period 2011-15 were classified as imported; for 54% of these cases the probable country of infection was another EU/EEA country.

Rubella Surveillance: 28 countries reported 2193 cases of rubella in 2015. Of those, 92.5% were reported from Poland. The incidence rate was below one case per million inhabitants for 25 countries and 16 countries reported zero cases. There were no rubella-associated deaths.

Between 2008 and 2015, 86% of rubella cases have been confirmed as IgM positive; however, these cases were classified as *probable* as per EU case definitions. Because of this discrepancy, efforts to harmonize the ECDC with WHO case definition for rubella are currently under review in a process that will involve formal approval by the EU commission. The working proposal is to include rubella IgM antibody detection (in addition to IgG serology, virus detection, and/or isolation) as a criteria for case confirmation.

Brainstorming and update on online accreditation check-list

Dr. Mick Mulders (WHO Headquarters)

To facilitate and streamline the accreditation program for measles and rubella, a web-based accreditation system similar to the already existing Polio LabNet system is in the process of being developed. Dr. Mulders presented the online platform used by the Polio LabNet, highlighting the features that will serve as the template for the new measles and rubella platform that is expected to be launched by the end of 2016

A major benefit of the Polio LabNet online platform is that all laboratories — global, national, and regional — can upload data to the same place. Each laboratory has the opportunity to provide information regarding laboratory space, staff, capacities, equipment, and publications, as well as a document center where protocols and SOPs can be shared. Repositories for proficiency testing, on-site/desk reviews, annual reports, and accreditation checklists are also integrated into the site. Global or regional lab coordinators can easily access these data, manage all the laboratories under their supervision, upload comments, and report directly to global headquarters.

Session 4 – Molecular diagnostics and epidemiology

Chair: Prof. Dr. Claude Muller

Process and results of 1st molecular PT (mPT) exercise in EUR

Dr. Oliver Donoso Mantke (INSTAND e.V.)

INSTAND e.V is an external quality assurance (EQA) provider based in Düsseldorf, Germany. They provide more than 350 EQA programs, 68 of which are virology-based, to international and national

participants in more than 80 countries. In 2015, the first molecular proficiency testing (mPT) exercise for measles and rubella in the WHO European Region was carried out in collaboration with NRLs in more than 28 countries. These first WHO/EURO- INSTAND EQA schemes for molecular detection of measles and rubella viruses were carried out by the INSTAND EQA advisor and assistant EQA advisor, Prof. Heinz Zeichhardt and Oliver Donoso Mantke. The mPTs were organized in close cooperation with WHO/Global VPD Laboratory Networks, Dr. Mick N. Mulders, WHO EURO/VPI/DCH, Dr. Myriam Ben Mamou, as well as the Robert Koch Institute/Regional Reference Laboratory WHO, Europe, Prof. Dr. Annette Mankertz and Dr. Sabine Santibanez. Dr. Donoso Mantke reported on the process and results from this first round of testing and provided recommendations going forward.

The goal of the EQA program was to assess the performance of laboratories that are routinely conducting molecular detection and characterization of measles and rubella viruses. The mPT testing scheme focused on three test categories: 1) genome detection 2) genotyping, and 3) sequence quality. The latter was discussed in detail by Dr. Santibanez.

For the respective virus, each laboratory was sent an FTA panel containing three samples of chemically inactivated virus plus one blank disk containing mock infected cell lysate. The measles panel contained undiluted B8, B3, and D4 specimens. The rubella panel contained two identical viruses of genotype 2B, and one specimen of genotype 1G (also undiluted).

Measles results (as of 14 March 2016): 32 laboratories participated in measles mPT and all were able to positively detect the viruses using either end-point or real time PCR assays. 25 laboratories sequenced the viruses and all were able to correctly identify the genotypes as B8, B3, and D4.

Rubella results (as of 14 March 2016): 30 laboratories participated in rubella mPT. 29 laboratories were able to positively detect the viruses using end-point or real time PCR assays. 88.2% of the laboratories were able to correctly identify the rubella genotype.

As agreed by the above mentioned cooperation partners, minimal requirements for successfully passing these first WHO/EURO- INSTAND EQA schemes for molecular detection of measles and rubella viruses were correct reporting of qualitative results (positive or below level of detection) for the whole set of panel members for the respective mPT. Those laboratories having performed genotyping had to report the correct genotypes for all positive samples of the respective mPT. The sequence quality for each virus containing sample was only considered for orientation, but not evaluated for certifications. The meeting members decided for the next PT rounds that 100% congruence with the target sequence will be a prerequisite for obtaining a certificate of successful participation.

Overall, the first mPT exercise was successful and will serve as the reference for the next rounds of proficiency testing both regionally and globally. In the ensuing discussion, it was decided that INSTAND e.V. and CDC will collaborate on harmonizing the mPT instruction sheet, the check-list of required information to be provided with the report, and the criteria and stringency of their assessment. Furthermore, they will work in collaboration with PHE and CDC to propose suggestions for a mechanism of online molecular EQA result reporting, including sequence submission to a dedicated MeaNS/RubeNS demo website.

Measles and Rubella Molecular PT in EUR: First experiences & genotyping results

Dr. Sabine Santibanez (RRL Berlin)

Dr. Santibanez reported on the quality and completeness of measles and rubella sequences from the INSTAND e.V. cooperation described in the previous section.

For both measles and rubella, a sequence was determined to be correct if it was 100% identical to the reference sequence — i.e., deviations/gaps/additions/ambiguities were not tolerated. Furthermore, the sequence should be complete, encompassing the entire sequencing windows for measles (N-450) and rubella (E1-739). If the sequence was longer than the recommended sequencing window, yet contained the correct fragment, it was still determined to be correct in these evaluations; however, this will likely change in the future as testing will become stricter.

If the sequences were incorrect, however, there was a large diversity of errors. Incomplete or ambiguous bases in one or more positions, a large number of nucleotides missing (up to 100, in one case), or the wrong frame or window were the major issues. Some countries did not know the correct measles nomenclature when reporting their results or had included long, unnecessary sequences.

Despite these issues, the results have been largely positive from the first round of molecular PT. 25 laboratories provided sequence data for all three measles samples, of which 76% of them reported the correct sequence; 17 laboratories provided sequence data for all three rubella samples, of which 88% reported the correct sequence. Of the countries who participated in both measles and rubella genotyping, 69% sequenced all six samples (both rubella and measles) correctly.

Update on last mPT exercise, US CDC

Dr. Bettina Bankamp (GSL United States)

In 2015, the US CDC more than doubled the number of participants (from 22 to 49 laboratories), including all RRLs and some selected NRLs, for its Molecular Proficiency Testing Program for measles and rubella. Dr. Bankamp discussed the challenges and successes they experienced, as well as the modifications they will make for the next round of proficiency testing in 2016.

A proficiency panel was sent to each of the 49 laboratories, containing four FTA[®] discs with inactivated measles or rubella viruses (as well as one blank disc containing no virus). Unlike 2014, backup panels were not provided in this round — re-testing required shipping an additional panel upon request. The laboratories were asked to test the panels using molecular assays that they *routinely* use, such as end-point PCR, RT-qPCR, genotyping RT-PCR and sequencing. Results were to be emailed to the Regional Coordinator, the Global Coordinator and CDC within six weeks using the Proficiency Panel Report form. In addition to reporting results, the form contains fields for summarizing the methods and a request for supporting data (such as images of agarose gels, sequencing files, and phylogenetic trees).

The criteria for passing the proficiency test were the following: correctly detect measles or rubella RNA (or negative reaction) in all of the samples with no false positive results; include adequate positive and negative controls on PCR reactions; correctly identify the measles or rubella genotypes in each positive sample; amplify and sequence the entire sequencing windows for measles (N-450) and rubella (E1-739) and have no more than one or two nucleotide errors. Laboratories were sent a Feedback Form indicating if they passed, must re-rest (due to minor errors), or failed (with significant errors).

Results have been largely positive from 2015. Of the laboratories that have submitted reports, most have produced high quality sequences with few problems in detection (only 2 false positives) and all

have correctly identified the genotypes. The turnaround time for submitting reports was between 1-12 weeks (average 5.5 weeks) and the distribution of the panels was more streamlined this year than the last due to the involvement of the Regional Coordinators in distributing the panels to their respective laboratories.

Ten reports are still outstanding and the results from ten countries are still pending due to re-testing requests. One country has failed for both measles and rubella, requiring that they receive additional training. Additional issues they saw include sending the wrong report form or file format and missing or poor quality supporting data, including: lack of positive controls on the gels and/or labeling issues, low quality images, missing C_t values, misalignment of phylogenetic tree/wrong branch length, incorrect sequencing window, using GenBank IDs for reference strains, and/or a high number or errors in the sequence.

Going forward, the Proficiency Panel Report form could be improved by adding a more detailed description of the required supporting data and acceptable file formats. Backup panels may also be sent to the laboratories at the onset of the study to expedite re-testing, if required. Regional Coordinators may be tasked with keeping track of panel delivery dates, sending the laboratories reminders to expedite reporting, ensuring that reagents are available before distribution of panels, and monitoring the age of the kits and methods used (since eight laboratories listed older lot numbers or older versions of methods). Options such as of web-based reporting, submission to MeaNS/RubeNS, determining named-strains, and combining measles and rubella in one panel are also under consideration.

The way forward to harmonize mEQA

Group Discussion facilitated by Dr. Mick Mulders (WHO headquarters)

A molecular External Quality Assessment (mEQA) program has been recently developed by CDC and rolled out in the Global Measles Rubella Laboratory Network (GMRLN) in collaboration with INSTAND e.V. The goal of the program is to assess the performance of the GMRLN laboratories that are routinely conducting molecular detection and characterization of measles and rubella. Furthermore, like the serology proficiency test program run on behalf of WHO by VIDRL, the mEQA program serves an additional educational role, where corrective action is initiated based on underperformance, including additional training. In the ensuing discussion, meeting participants discussed how to harmonize the mEQA schemes underway in the GMRLN laboratories, and agreed upon the following recommendations.

GMRLN laboratories should enroll in mEQA schemes strictly corresponding to their routine work (end-point and/or real time RT-PCR/sequencing for genotyping of measles and/or rubella). The goal of mEQA is to evaluate assays conducted on a routine basis; hence, laboratories that are not regularly performing any of these assays should not participate. CDC is providing practice panels to those laboratories that want to retain their expertise in conducting such assays. Laboratories not fulfilling the above requirements on a routine basis can be referred to regular EQA programs of recognized EQA providers.

INSTAND e.V. and CDC will collaborate to harmonize mEQA programs regarding reporting, and updating the instruction sheet, the check-list of required information to be provided with the report, and the criteria and stringency of their assessment. Strict evaluation of sequence quality for the next round will be implemented (i.e., zero nucleotide error as well as correct length). A dedicated side-meeting to harmonize the global mEQA program will take place at the GMLRN14 meeting in Geneva at the end of June, 2016.

PHE, CDC and INSTAND e.V. will also propose suggestions for a harmonized mechanism of online mEQA result reporting, including sequence submission to a dedicated MeaNS/RubeNS demo website. The working group will report back at the GMRLN14 meeting and present a mockup of such website. Furthermore, INSTAND e.V. will consider classification of mEQA as non-infectious for easier international shipment. Verification of non-infectivity of measles and rubella viruses, respectively, will be performed by Robert Koch Institute Berlin.

Molecular epidemiology of measles and rubella in EUR, 2015

Dr. Kevin Brown (GSL United Kingdom)

On the global level, 5362 measles sequences were submitted to the measles nucleotide surveillance database (MeaNS) in 2015. 16% of these sequences (n = 865) were submitted from the European Region. The predominant genotypes reported for 2015 were D8, comprising 85% of all sequences, mostly reported from Germany (29%) and the Russian Federation (28%); B3 came in second (11%), with the United Kingdom reporting the largest fraction. D4, D9, and H1 — likely imported from China — formed a small percentage of submissions. Importantly, these percentages do not accurately represent the true global distribution of the viruses due to strong reporting bias.

A reduction in the diversity of circulating measles strains continues to be observed over the past few years. Rostov on Don and Chui were the major named-strains for D8, with Germany and the Russian Federation reporting the most sequences, respectively. Kansas and Harare were the major named-strains for B3, primarily reported from Germany and the UK.

Submission delays were of significant concern in 2015. Users have been asked to submit within two months, yet the average time has been 19.5 weeks (range: 2.8 – 37 weeks). Furthermore, vaccine genotype A continues to be entered into the database. To help remedy this, instructions on how to enter data will be offered in other languages.

In contrast to the situation with measles, few countries are submitting genomic sequence information on rubella cases to the rubella nucleotide surveillance database (RubeNS). In 2015, only nine sequences were submitted in total, with 2B predominating.

Vaccine specific RT-PCR for measles (MeVA)

Dr. Alberto Severini (Public Health Agency, Canada)

A rapid, high throughput RT-PCR that specifically detects measles vaccine genotype A (MeVA) has been developed at the Public Health Agency of Canada (PHAC) led by Dr. Severini. The MeVA RT-PCR method will allow laboratories to quickly discriminate between wild type infections and vaccine reactions without the need for sequencing of the standard N-450 sequencing region, which may take several days.

The MeVA RT-PCR has been tested on the Roche Lightcycler 480 platform, showing a specificity of 100% and a sensitivity of approximately 97%. US CDC has independently tested the MeVA RT-PCR on the Applied Biosystems 7500 platform with the same results. The choice of kit is critical to the method, with only the QuantiTect RT-PCR Kit produced by Qiagen showing optimal performance and specificity. RNA from five currently used vaccine strains, AIK-C, CAM-70, Edmonston-Zagreb, Moraten, and Shanghai-191 have all been positively detected using the new method.

It is recommended that the MeVA qPCR should be performed in parallel with the standard MeV qPCR for every suspected vaccine case, in addition to every suspected case in children of vaccination age. Vaccine positive cases can therefore be reported immediately and later confirmed by routine sequencing. Multiplexing of the two methods will increase the efficiency of the approach and is currently in the validation phase at the PHAC.

Implementing the measles vaccine-specific RT-PCR at the RRL Berlin

Amy Lüdde, MSc (RRL Berlin)

Ms. Lüdde has been working on validating and implementing the measles vaccine-specific MeVA RT-PCR developed by the Public Health Agency of Canada into the RRL Berlin's routine diagnostics.

Investigations into whether the Invitrogen Superscript III RT-PCR kit could be optimized for use with the new method — since it is already being used in their accredited diagnostic RT-PCR for measles — was of primary focus in the early stages of the research. Different primer-probe concentrations, cycling conditions, and reagent concentrations were tested, all revealing suboptimal performance for the Invitrogen kit — the major concern was the high number of false positive samples of wild-type genotypes B3, D8 and D10.

Based on these evaluations, it was determined that MeVA RT-PCR implementation will proceed using the QuantiTect RT-PCR Kit produced by Qiagen, recommended by Dr. Severini and colleagues. The kit and the protocol are currently being fully validated and it is anticipated that the protocol will be rolled out into the RRL's routine diagnostics by August, 2016.

US CDC Update: New Approaches and Technologies/Measles

Dr. Paul Rota (GSL United States)

The US CDC is working on validating the Luminex[®] assay to determine its suitability for use in seroprevalence studies. They will test a large panel of serum samples from previous seroprevalence and vaccine studies tested by EIA and/or neutralization assays; additionally, they are developing a protocol for standardizing the Luminex[®] beads (e.g., with regard to performance, consistency, lot-to-lot variation, costs, sustainability, and scale up). Their goal is to pilot the assay at an RRL within the WHO LabNet before being rolled out.

Though still in the evaluation phase, tests thus far have demonstrated that the two secondary antibodies — one recognizing the H+L chains and one recognizing the Fc portion of IgG — performed nearly equally well for measles and rubella in the Luminex[®] assay. Furthermore, measles and rubella antigens were successfully conjugated to generate new Luminex[®] bead stocks.

The US CDC is working on validating and eventually transitioning their viral surveillance activities from Sanger sequencing to next generation sequencing (NGS) methods in a project called Advanced Molecular Detection within the Division of Viral Disease (DVD). In 2015, more than 500 viral genomes were obtained by NGS within Division, of which measles and rubella sequences were also examined. The plan for NGS is to further implement and document quality practices for NGS, including the development of SOPs, laboratory protocols, and instrument validation procedures. Once standardized laboratory and bioinformatics workflows have been established, they aim to formalize pipelines for moving the technology out to higher performing state laboratories and international partners for piloting and hands-on training. An open web portal is also under development such that laboratories that want to work on NGS, but do not have all the bioinformatics resources available, can do so.

US CDC Update: New Approaches and Technologies/Rubella

Dr. Joseph Icenogle (GSL United States)

In an effort to improve global rubella molecular surveillance, the rubella team at the US CDC is researching a method for obtaining rubella virus genotypes from archival sera or dried blood spots using real-time assays. The method involves selecting IgM positive sera, extracting the RNA, and using real-time RT-PCR for detection. The positive samples are then sequenced using nested-set amplification. In one of the studies they published on archival sera from 11 countries, they found that 18% of the samples were positive following real time RT-PCR, and 44% of these positive samples could be amplified and sequenced. Two additional papers on this method are currently in press or under final review, also showing a success rate of between 10-20%. It was underscored that there is a widely accepted need for obtaining additional representative data on currently circulating rubella genetic lineages — especially in countries with low incidence — therefore, laboratories will be encouraged to utilize this method to improve their molecular surveillance.

Several laboratories in the network have had issues with the sensitivity of the rubella genotyping assay, due in part to the low copy numbers from samples of rubella cases. The rubella team determined that changing to a new enzyme kit (Invitrogen SuperScript III High Fidelity kit) could improve sensitivity by approximately 10-fold for both rubella isolates and clinical samples. To validate its increased sensitivity, three MR LabNet laboratories were sent updated protocols for testing and confirmation; however, only one laboratory has tested the protocol and confirmed results thus far (for rubella isolates only and no clinical samples). The CDC has yet to obtain results from the other laboratories, especially those testing clinical samples.

An improved diagnostic and duplex RT-qPCR for detecting rubella viral RNA has been developed in collaboration with Dr. Min-Hsin Chen. The new assay has been tested with sera, oral fluid, throat swabs, and FTA® card specimens from Peru, DRC and Romania. Results have shown that 81% of oral fluid specimens collected within three days of rash onset have less than 100 copies/uL of rubella RNA per extraction. Furthermore, they have also found that 28% of IgM positive serum specimens collected within three days of rash onset were positive using this improved assay.

New training approaches have been implemented over the past year at the rubella laboratory. Epidemiologists have been integrated into the agenda of the training courses which has been valuable in informing participants about CRS case classification, recording, and reporting. Providing participants with mock cases and mock specimens has fostered more engagement and learning, especially because they can see the hands-on relevance. Time will tell if the lessons learned will be brought back to their own laboratories.

Session 5 – Serology

Chair: Dr. Paul Rota

Development and validation of control serum panels

Dr. Tamara Mamaeva (RRL Moscow)

In 2015, the Moscow RRL developed control serum samples and panels for IgM/IgG EIA tests for measles and rubella. In addition to determining the criteria on how to store and reconstitute dried serum samples, they tested different ELISA kits from different suppliers, including Vector, Siemens and Ecolab, in an effort to determine the optical density and the serum dilution required for each kit based on their respective cut off values

Dr. Mamaeva reported that internal quality control (IQC) is being uniformly implemented throughout the Russian Federation and the NIS. To further implement IQC and EQA measures in national and sub-national laboratories in the region, the RRL has conducted five workshops (one in Tajikistan, one in Belarus and three in the Russian Federation) and trained 29 virologists in both theory and practical applications.

Joint studies of IQC are currently underway with manufactures of the Ecolab kit in order to test the kit's stability. To further develop and validate IQC methods that are currently underway within the region, the RRL will be setting up workshops in collaboration with the WHO.

Harmonization of IgM retesting exercise

Group discussion facilitated by Dr. Judith Hübschen (RRL Luxembourg)

The IgM retesting form has been harmonized and adopted by RRLs following 2015 recommendations.

Meeting participants agreed that the new retesting form should include kit-specific sheets to better accommodate retesting results from laboratories not using Siemens kits, like the reporting form previously used for serology proficiency testing. Furthermore, it was decided that a more sensitive system should be applied for the retesting results assessment, for example taking differences in corrected delta OD values into account and not only qualitative results. RRLs will review PHE and AFRO approaches and discuss suitable options for the MR LabNet.

Meeting participants also discussed that cellular controls should be introduced for RT PCR, especially if this is the only method used for case classification. Thus, revising the accreditation check-list to include more stringent PCR prerequisites is under consideration.

Update on 2015 ETAGE statements and recommendations on serosurveys

Dr. Myriam Ben Mamou (WHO Regional Office for Europe)

The European Technical Advisory Group of Experts on Immunization (ETAGE) met on the 30th of September, 2015 in Copenhagen, Denmark. One session was devoted to gauging the feasibility and value of serosurveys in the context of verifying measles and rubella elimination in the European Region.

At this meeting, feedback from the MR LabNet was presented by Dr. Ben Mamou, Dr. Brown, and Dr. Jankovic. They covered topics comprising both laboratory and epidemiological perspectives including: sampling and implementation issues, the limitations in interpreting results, and the substantial technical and resource burdens associated with serosurveys. Furthermore, it was emphasized that good alternatives to serosurveys often already exist, including the presence of high quality epidemiological data or existing, yet not implemented, programmatic interventions.

Based on these concerns outlined by the MR LabNet, ETAGE came to the following recommendation:

Serosurveys should only be conducted when there is a paucity of existing data or indicators on a particular high risk population or geographical area, whereby targeting this group/area will help to solve a clearly defined public health issue.

The full meeting report can be found at <http://www.euro.who.int/en/health-topics/disease-prevention/vaccines-and-immunization/publications/2015/15th-meeting-of-the-european-technical-advisory-group-of-experts-on-immunization-etage>

US CDC update on Rubella serology update

Dr. Joseph Icenogle (GSL United States)

Dr. Icenogle provided four case studies highlighting the complexities and limitations involved with seroprevalence studies. The case studies revealed the following issues:

- The distribution of titers may be higher than the cutoff for a specific assay. Furthermore, assays are not as standardized as one would expect.
- There is not a stable distribution of serologic titers over time. Antibody titers are flexible in some individuals.
- Converting seroprevalence to immunity is a difficult task — losing antibodies does not necessarily mean that you are not protected.
- Interpreting seroprevalence depends on the studied population.

Update on POCT testing using oral fluid for measles IgM and IgG

Dr. Kevin Brown (GSL United Kingdom)

An improved point of care test (POCT) for detecting measles infections in the field is being validated at Public Health England (PHE). The on-site test has two components: collecting and extracting oral fluid (OF) using the newly developed Oralight collection device, followed by directly applying the OF onto a lateral flow device that can detect measles IgM or IgG antibodies in a matter of minutes.

A major advantage of the Oralight collection device is that OF from the gingival tissue can be immediately and easily extracted by hand into the unique Oralight vessel — instead of sending the specimen to a laboratory for extraction and testing (the current process required for the Oracol™ swabs that have been in use over the last two decades). The PHE has found that the sensitivity of the Oralight collection device is not as high as for the Oracol™ swabs, yet the concordance between both collection methods is strong, and its efficiency cannot be overstated. Furthermore, in addition to being inexpensive and easy to use, the new device has been proven to be safe, exhibiting no toxicity or leachability, and patients widely accept its use since it is non-invasive and painless. Based on these evaluations, the PHE recommends that the Oralight collection device replace the Oracol™ swabs in the field for POCTs.

After the OF is collected and extracted in the Oralight collection vessel, it can be inverted and used as a dropper to dispense the specimen onto the nitrocellulose membrane of a lateral flow device containing antibodies directed against IgG or IgM. After only 15 minutes of incubation at room temperature, the results can be read. Aside from visual inspection, an ESEQuant lateral flow reader can be taken into the field to measure the quantitative value of the tests (via reflectance traces) and to transfer the results in real-time. In a study from 2011, the lateral flow device showed a sensitivity of 90.0% and a specificity of 96.2% using oral fluids. A recent study using sera specimens from the

Brazilian surveillance system showed that the POCT had a sensitivity of 96%, a specificity of 100%, and concordance with the Siemens IgM ELISA assay of 96%.

The time it takes to obtain results using the improved POTC — from time of collection using the Oralight device to reading the results from the lateral flow device — is only 22 minutes. The benefits are clear: patients can be quickly informed of their status, vaccines can be administered on that day, and case reporting to NRLs and RRLs can be expedited directly from the field. Furthermore, RNA can be extracted from the POTC and genotyped, increasing the options for implementing high quality molecular surveillance, especially in remote areas.

Session 6 – Future plans & recommendations

Chair: Dr. Sabine Santibanez

Agenda and format of NRL meeting, MNE, June 2016

Group discussion facilitated by Dr. Myriam Ben Mamou (WHO Regional Office for Europe)

Meeting participants were asked to give suggestions and comments on the agenda and format of the next NRL meeting that will be held at the end of June 2016. The meeting will focus primarily on training sessions and teaching lectures in the first two days, with regional and global updates on the third day.

A key suggestion from the discussion was replacing the country presentations that took place last year with a more efficient poster session. It was proposed that each country could receive a “poster-template” facilitating the entry of important data, including a section for challenges that they may have encountered. To foster small group discussions and more direct interactions with laboratories that experience specific concerns, small break away sessions will be integrated into the agenda; participants with specific issues will be invited to give a ten minute presentation to open communication pipelines and opportunities for learning to all.

The training sessions and lectures will include topics such as the newly developed MLRDMS-2, maintaining high quality surveillance, diagnosing vaccine failure, understanding the Westgard rules, and informing participants on the changes in serology testing. Dr. Ben Mamou will be coordinating with all meeting participants to finalize the agenda and format.

Publications

The strategy for the publication of papers by the MR LabNet laboratories was discussed:

Prof. Dr. Mankertz has offered to coordinate a publication summarizing the molecular epidemiology of measles transmission in the European Region from 2012-2015. Data from regional verification processes and molecular epidemiology will be highlighted, including data from the Russian Federation-NIS and the UK. Because of the large amount of data, general patterns will be depicted using easy-to-understand graphics, including color-coded maps showing GT distribution, and not just phylogenetic trees. The paper will further highlight the reduction in measles and rubella diversity over the last three years, underscoring that some countries no longer report circulation.

Dr. Santibanez and Dr. Brown will draft a paper on the quality of surveillance within the Region to identify gaps in molecular surveillance. Discrepancies in the number of cases reported vs. data reported to MeaNS will be of primary focus.

Dr. Mulders is in the process of finalizing a submission to MMWR on the Global MR LabNet.

Dr. Donoso Mantke and Prof. Dr. Zeichhardt will draft a paper on "Experiences from the first WHO/EURO-INSTAND EQA schemes for molecular detection of measles and rubella viruses in comparison to national EQA schemes" with co-authorship of Prof. Dr. Mankertz, Dr. Santibanez, Dr. Mulders and Dr. Ben Mamou.

Recommendations

ACCREDITATION / EQA

IgM retesting: The retesting form has been harmonized and adopted by RRLs following 2015 recommendation and each RRL send specific instructions according to its retesting modalities.

1. The form needs to include kit-specific sheets to better accommodate retesting results from laboratories not using Siemens kits, like the reporting form previously used for serology proficiency testing.
2. A more sensitive system should be applied for retesting results assessment. RRLs to review PHE and AFRO approaches and discuss suitable options for EURO.

Molecular EQA (mEQA)

A molecular External Quality Assessment Program has been recently developed by CDC and rolled out in the Global Measles Rubella Laboratory Network in collaboration with Instand e.V. The mEQA program goal is to assess the performance of the GMRLN laboratories that are routinely conducting molecular detection and characterization of measles and rubella. Furthermore, like the serology proficiency test program run on behalf of WHO by VIDRL, the mEQA serves as an additional educational role, where corrective action is initiated based on underperformance, including additional training.

3. Scope : GMRLN Laboratories should enrol in mEQA schemes strictly corresponding to their routine work (end-point and/or real time RT-PCR / sequencing for measles and/or rubella). Laboratories that are not performing any of these assays on a routine basis should not be participating, since mEQA assesses assays conducted as a routine activity. CDC is providing practice panels to those laboratories that want to retain their expertise in conducting such assays.
4. Process harmonization: Instand and CDC to collaborate to harmonize mEQA programmes regarding sample characteristics, reporting, instruction sheet, check-list of required information to be provided with the report, criteria and stringency of result assessment. Apply strict evaluation of sequence quality for next round (zero nucleotide error). A dedicated side-meeting to harmonize the global mEQA program is being scheduled during the GMLRN14 meeting in Geneva on Friday 24 June.
5. Online result reporting: PHE, CDC and Instand to propose suggestions for a harmonized mechanism of online mEQA result reporting, including sequence submission to a dedicated MeaNS/RubeNS shadow/demo website. The working group will report back at GMRLN14 (side-)meeting and present at best a mockup of such website.
6. Instand e.V. to consider classification of mEQA as non-infectious for easier international shipment (CDC to share protocols)

Other accreditation issues

7. Network Laboratories are urged to comply with timeliness and completeness reporting requirements to MeaNS and RubeNS (within 2 months of specimen receipt) and share all sequences generated by or on behalf of the laboratory.
8. WHO/EURO to expedite MRLDMS.2 finalization, RRL to collaborate for piloting MRLDMS.2, WHO to provide access to demo version for GSL / RRLs to test the platform.
9. To facilitate and streamline the accreditation program by developing and implementing a web-based accreditation system similar to the already existing polio labnet system.
10. In order to maintain the accuracy and sensitivity of detection, it is required to introduce cellular controls when RT-PCR is used for case classification. Consider the revision of the accreditation check-list to include more stringent PCR prerequisites.

MOLECULAR DETECTION / SURVEILLANCE

11. Rubella genotype data is scant and there is an urgent need to obtain additional representative data on currently circulating rubella genetic lineages. The US-CDC RT-PCR protocol for rubella genotyping directly from sera has proven to be somewhat successful, with a success-rate of about 10-20%. To enhance molecular surveillance of rubella, laboratories are encouraged not only to increase their efforts in obtaining samples for rubella genotyping, but also to consider using this CDC protocol. Rubella positive sera forwarded for confirmatory testing to the supervisory RRL/GSL should be screened routinely for rubella virus RNA and sequenced where possible. Other opportunities should be explored to enhance molecular surveillance of rubella e.g. WHO / RRL Berlin / RVC / ECDC country missions to explore the availability of suitable rubella specimens in NRLs for genotyping in RRLs/GSL.

VERIFICATION OF ELIMINATION

It is part of NRL's responsibilities to provide NVC with verification quality virologic surveillance and guidance to increase integration of epidemiological data with sequence information to inform RVC on virus transmission pathways.

12. NVC should set up an advisory mechanism providing expert virology advice in the preparation of NVC annual updates to RVC, including laboratory representation.
13. NRLs are urged to use MeaNS NRL listing function to fill out outbreak table in ASUs.
14. NRL meeting in June 2016 should provide a training opportunity to NRLs on how to use of this functionality.
15. Discordances between ECDC and WHO disease case definitions are sometimes challenging for countries to classify their cases. ECDC and WHO to collaborate to harmonize measles, rubella and CRS case definitions and revise surveillance guidelines as appropriate.

CAPACITY BUILDING / TRAINING

16. Training approach for EUR has moved to a "second-generation", not delivering generic hands-on trainings but tailored / individual trainings based on identified needs, possibly integrated in regional labnet meetings (i.e. sequence data management).

17. Training development to adopt a concerted approach, optimize the use of already existing resources, e-learning, case-based oriented workshops, involvement of epidemiologist when appropriate.
18. Training / capacity building evaluation mechanism to be developed.

PUBLICATIONS

It was agreed to draft one global and two regional papers:

19. Molecular epidemiology in EUR, 2012-2015 (A Mankertz to coordinate)
20. Quality of surveillance / identify gaps in molecular surveillance (S. Santibanez / K. Brown)
21. Global LabNet in MMWR (MM)

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.

Member States

Albania
Andorra
Armenia
Austria
Azerbaijan
Belarus
Belgium
Bosnia and Herzegovina
Bulgaria
Croatia
Cyprus
Czechia
Denmark
Estonia
Finland
France
Georgia
Germany
Greece
Hungary
Iceland
Ireland
Israel
Italy
Kazakhstan
Kyrgyzstan
Latvia
Lithuania
Luxembourg
Malta
Monaco
Montenegro
Netherlands
Norway
Poland
Portugal
Republic of Moldova
Romania
Russian Federation
San Marino
Serbia
Slovakia
Slovenia
Spain
Sweden
Switzerland
Tajikistan
The former Yugoslav
Republic of Macedonia
Turkey
Turkmenistan
Ukraine
United Kingdom
Uzbekistan

World Health Organization Regional Office for Europe

UN City, Marmorvej 51, DK-2100 Copenhagen Ø, Denmark

Tel: +45 45 33 70 00 Fax: +45 45 33 70 01

Email: eurocontact@who.int

Website: www.euro.who.int