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MEETING REPORT

14TH MEETING OF THE MEASLES / RUBELLA REGIONAL REFERENCE LABORATORIES OF THE WHO EUROPEAN REGION

14 - 15 MARCH 2019, ESCH SUR ALZETTE - LUXEMBOURG



ABSTRACT

The 14th meeting of the Measles/Rubella Regional Reference Laboratories (RRL) of the WHO European Region was held in Esch-sur-Alzette, Luxembourg on 14- 15 March 2019. This report consists of a summary of the presentations given by laboratory representatives and technical experts, including updates on the status of the WHO measles/rubella (MR) programme, proficiency testing, and the MR elimination verification process, and lists the recommendations that resulted from the discussions.

KEYWORDS

COMMUNICABLE DISEASE CONTROL

DISEASE ELIMINATION

SURVEILLANCE

EPIDEMIOLOGY

IMMUNITY

LABORATORIES

ACCREDITATION

VERIFICATION

MEASLES

RUBELLA

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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
CRI	congenital rubella infection
CRS	congenital rubella syndrome
DI	discriminatory index
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
ES	enhanced (active) surveillance
EQA	external quality assessment
EVAP	European Vaccine Action Plan
EU	European Union
GMRLN	Global Measles and Rubella Laboratory Network
GSL	Global Specialized Laboratory
HH6	human herpesvirus type 6
IQC	internal quality control
IgG	immunoglobulin G
IgM	immunoglobulin M
LabNet	European network of measles/rubella laboratories
NAL	nationally accredited laboratory
MCV	measles-containing vaccine
MeaNS	Measles Nucleotide Surveillance Database
MeV	measles virus
MF-NCR	measles virus non-coding region between matrix and fusion genes
MR	measles/rubella
MRLDMS	measles and rubella laboratory data management system
N-450	measles virus genotyping region: 450 nucleotides C-terminus of nucleoprotein gene
NGS	Next Generation Sequencing
NIS	newly independent states
NL	national laboratory
NRL	national reference laboratory
NVC	national verification committee
OF	oral fluids
PCR	polymerase chain reaction
PHE	Public Health England
PRN	plaque reduction neutralization
PP	proficiency panel
PT	proficiency test
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 Database
RuV	rubella virus
RVC	Regional Verification Commission for Measles and Rubella Elimination
SAGE	Strategic Advisory Group of Experts on Immunization
SNL	subnational laboratory
SIA	supplemental immunization activity
Tessy	European Surveillance System
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America

Executive summary

The 14th meeting of the Measles/Rubella Regional Reference Laboratories (RRL) of the WHO European Region was held in Esch-sur-Alzette, Luxembourg on 14- 15 March 2019.

Representatives of the following institutions/laboratories attended the meeting:

- European RRLs: Gabrichevsky Institute (Moscow), Luxembourg Institute of Health (Luxembourg) and Robert Koch Institute (Berlin);
- Global specialized laboratories (GSLs) at Public Health England (London) and the United States Centers for Disease Control and Prevention (CDC) (Atlanta);
- European Regional Verification Commission for Measles and Rubella Elimination (RVC);
- WHO headquarters and Regional Office for Europe.

During the meeting, updates on the status of the WHO measles/rubella (MR) programme, proficiency testing, and the MR elimination verification process were presented and discussed. A set of recommendations agreed during the meeting is included in Section 3 – Recommendations.

1. Introduction

The European network of Measles/Rubella (MR) laboratories (LabNet) was set up in 2002. It is composed of 73 laboratories arranged in a tiered structure coordinated by the WHO Regional Office for Europe. The role of the LabNet is to ensure and coordinate a high-quality laboratory diagnosis service. The global specialized laboratory (GSL) in London and three regional reference laboratories (RRLs), in Berlin, Luxembourg and Moscow, supervise proficiency testing and assay implementation in national reference (NRL) and sub-national (SNL) laboratories.

As the WHO European Region progresses towards elimination of measles and rubella, good surveillance and effective testing of potential cases becomes increasingly important. The scope of this meeting of the European MR RRLs was to share recent information on LabNet's achievements, challenges and research in laboratory aspects of measles and rubella surveillance and on laboratory contribution to the verification process in the European Region.

This report consists of a summary of the presentations given by laboratory representatives and technical experts and lists the recommendations that resulted from the exchanges and discussions that took place during the meeting.

The Regional Office is grateful to Luxembourg health authorities and to the Luxembourg Institute of Health in Esch sur Alzette for hosting this meeting.

2. Sessions of the meeting

Dr Jean-Claude Schmit (Luxembourg Ministry of Health)

The Government of Luxembourg is very concerned about the high incidence of measles in Europe. In Luxembourg, there is a high level of compliance with the vaccination schedule, confirmed by surveys carried out every 4 to 5 years. So far, the anti-vaccination movement has had limited impact in Luxembourg, and the elimination status has been maintained.

Dr Markus Ollert (LIH Department of Infection and Immunity)

The Luxembourg Institute for Health (LIH) is multi-disciplinary, encompassing fields such as oncology, immunity, inflammation and infectious disease. Professor Claude Muller promoted the LIH as a Regional Reference Laboratory (RRL) of the LabNet, and now gives support to other WHO Member States in the implementation of high-quality surveillance systems and of strategies for the elimination of measles and rubella in the European Region and in other regions.

The LIH, and the Luxembourg RRL in particular, have established collaborations worldwide to assist developing countries in fighting the double burden of infectious and lifestyle-associated diseases through the transfer of knowledge. This activity has been associated with a rapid expansion of the department since 2014.

Session 1 – Regional and global updates

Chair: Dr Judith Hübschen (RRL Luxembourg)

1.1. WHO Regional Office: status of LabNet and elimination of measles and rubella in European Region

Dr Dragan Jankovic (WHO Regional Office for Europe)

Cooperation between laboratory services and epidemiologists in charge of measles and rubella programmes in countries is being actively promoted by the Accelerated Disease Control team of the Vaccine-preventable Diseases and Immunization programme, Regional Office. The RRLs and Global Specialized Laboratory (GSL) in London are essential in assuring sufficient capacity of the laboratories in the Region. Although several of the objectives in the Region have been accomplished, such as sustaining the polio-free status, supporting countries in the introduction of new vaccines, and ensuring that immunization programmes are financially sustainable, the elimination of measles and rubella (MR) is yet to be achieved and vaccination coverage lags below targets in many countries.

The level of coverage of the second dose of measles-containing vaccine (MCV2) is the highest ever recorded in the Region, at 91% in 2018. The coverage of the first dose of MCV (MCV1) is at 95%. Ukraine was the country with the highest number of measles cases in 2018 with 63% of all cases reported in the Region that year.

The profiles of susceptible populations vary between countries, with countries where vaccination rates improved more recently seeing more cases in older populations, while those with falling coverage reporting more cases in younger individuals. In some countries a combination of these two factors can be observed, while in others continued low vaccination rates lead to the distribution of measles cases relatively evenly through all age groups.

The increased incidence of measles cases, with outbreaks spreading across borders in 2018 has forced countries to review their procedures on dealing with suspected measles cases. The high incidence of measles among adolescents, adults, and even healthcare workers (HCWs) in some cases highlights immunity gaps that must be targeted by vaccination campaigns. In order to decrease nosocomial

transmission, suspected measles cases should not be hospitalized unless clearly indicated, and particularly not when suboptimal infection control measures are in place. When a patient with measles is hospitalized, they should be isolated, and immunization should be required for all HCWs in contact with them. Children under one year old can only be protected if the other members of their family unit are vaccinated. As an urgent measure, an extra dose of vaccine at 6 or 9 months of age could be administered during outbreaks.

The number of measles cases in Georgia is currently in sharp decline, and control measures are coming into effect in Azerbaijan, Kazakhstan, and Ukraine, with additional vaccination campaigns and facilitation of access to immunization.

The situation for rubella is different, in that it is unclear how good surveillance systems are in some countries, but the number of reported cases is decreasing overall. Rubella may have already been eliminated in several countries, however verifying it is challenging in the absence of well documented case investigation and appropriate classification.

Many countries are adopting policies in response to vaccine hesitancy and the increase in measles cases. Sustaining high vaccination coverage is becoming a central concern to the European Union Council. Main challenges for the coming years include mobilizing resources to support middle-income countries, and in understanding and addressing reasons for vaccine refusal and hesitancy.

Dr Myriam Ben Mamou (WHO Regional Office for Europe)

There are currently 73 laboratories (71 testing for both measles and rubella, 1 only for measles and 1 only for rubella) in 50 countries (Member States of the WHO European Region), with those in Montenegro and Switzerland having been accredited for the first time in 2018. The annual accreditation desk-review is underway with re-testing between February and April 2019 at the RRLs. The deadline for accreditation checklists was at the end of February, and the reports to the national verification committees are to be submitted by 15 April.

Many accreditation visits were conducted in 2018 with the members of the RRLs and GSL (London). In 2019 (with 2017 data), 72 laboratories were fully accredited for serology, and one was provisionally accredited. 33 of 34 laboratories received accreditation for measles RT-PCR, 26 out of 30 obtained measles sequencing accreditation, 29 of the 32 laboratories that applied for rubella RT-PCR accreditation were successful, while 17 of 19 obtained rubella sequencing accreditation. 67 laboratories of 72 obtained a full score in part 1 of the measles serology proficiency testing, while 57 achieved a full score in part 2 of the scorecard. 50 of the 72 laboratories were given a final score of 100%. The majority of laboratories (71 of 72) that conducted rubella serology proficiency testing achieved a full part 1 score and 57 obtained 100% in part 2. All 73 laboratories obtained accreditation in measles and/or rubella serology. However, some laboratories continue to test a limited number of serology samples; and there are issues with compliance with RRLs' retesting schemes, and with the shipment and testing of a variety of sample types. Commonly used specimen types in the Region include liquid sera, dried serum spots (DSS), dried blood spots (DBS) and oral fluids (OF).

37% of sera from measles-suspected cases tested for measles IgM were positive in 2019, up 4% from the previous year. Only 2% of sera investigated for rubella IgM were positive in 2018 and 2019, as the greater portion of rubella serology testing results from systematic testing rather than testing of

suspected cases. One laboratory failed to show evidence of use of an in-house control, and some laboratories used expired kits.

All laboratories that participated in the molecular external quality assessment (mEQA) and submitted their results so far (n=34) have passed all components of the test. Seven countries that reported measles cases did not submit sequences to the measles nucleotide surveillance database (MeaNS). 40 have reported cases and submitted sequences to MeaNS, although 26 sequenced less than the recommended 80% of chains of transmission. Six countries reported neither measles cases nor sequences.

The accreditation checklist has been revised to harmonize sections, remove duplications and include a section about documents and records control. The checklist now includes ten essential criteria for SNLs and NRLs. The RRLs and GSL do not have re-testing results and need to achieve a minimum score of 90%, which is 10% higher than the score required for the remaining laboratories.

The WHO Regional Office continues to help countries build capacity and expertise through the organization of LabNet meetings, include training sessions, and tailored hands-on training during accreditation visits and in RRL premises. There has been an increased workload at the Regional Office to supply network laboratories with different reagents and panels, because of the switch of IgM enzyme immunoassays (EIAs) to Euroimmun, the broader use of FTA[®] filter paper, coordination of serology and molecular EQAs and the growing demand for US CDC molecular reagents and practice panels.

A new contractor is taking over the development of the second version of the measles rubella laboratory data management system (MRLDMS2). Finalization of the platform will take into account WHO headquarters' plans to develop the new information system WISE.

The LabNet is expanding, with two new participant countries, and it demonstrates high levels of proficiency, performance and molecular expertise. Concerns are mainly related to the use of internal quality controls (IQC), procurement and shipment of reagents, the transition period due to the serology reagents change, the reliance on RT-PCR for exclusion of cases in some countries, and continuing training needs due to high staff turnover.

1.2. WHO global update on the global Measles and Rubella Laboratory Network and measles and relimination programme

Dr Mick Mulders (WHO headquarters)

The global elimination of measles appears more distant than it was 10 years ago, in great part due to a plateau in vaccination coverage of MCV1. The MCV2 coverage is increasing, but still below the recommended level of 95%. The increase in the number of cases recorded in recent years may also reflect improvements in the sensitivity of surveillance systems in the last 10 to 20 years. Despite the drawbacks, there were 95% fewer measles deaths in 2017 than in 2000, with an estimate of 21 million deaths prevented by vaccination in this period.

The WHO Americas Region is the region reporting the fewest cases of measles and rubella. 97% of the countries in this Region have achieved measles elimination and all have managed to eliminate rubella. 70% of countries in the European Region have achieved measles and rubella elimination. There has been a dramatic reduction in the number of measles cases in the Western Pacific Region, with few

measles virus (MeV) genotype H1 sequences being reported, and importations of genotypes B3 and D8 now being detected. 33% and 19% of countries in this Region have achieved measles and rubella elimination, respectively. The Southeast Asia Region is moving towards elimination, with better surveillance and a strong decrease in the number of measles cases. 18% of countries managed to eliminate measles. Military conflicts in the Eastern Mediterranean Region have led to increases in the number of measles cases in that Region. Regional verification commissions have now been established in the Eastern Mediterranean and African regions.

When the number of cases of measles decreases and vaccination rates improve, waning immunity and secondary vaccine failure will need to be considered more often. Many new technologies and assays are being deployed that will benefit and alter the measles and rubella programme. Micro-array patches containing measles vaccine could facilitate immunization and increase coverage. Point of care tests (PoCT) will make diagnosis instant and portable.

There are now 714 laboratories in the WHO global MR LabNet, including 4 GSLs, 14 RRLs, 180 NRLs, and 516 SNLs. The China CDC is now accredited as a GSL. In 2018, a new laboratory manual was made available online, a new accreditation checklist was rolled out, and a 3rd round of the mEQA and the 19th round of the serology EQA schemes were conducted. A kit comparison study is being carried out ahead of the transition to new serology kits due to the interruption in the supply of Siemens manual serology kits. Several serology workshops were conducted and new guidelines on the next generation, extended window and whole generation sequencing were also published last year.

Work on the update of the EQA and mEQA websites is ongoing and a second version of MeaNS and Rubella Nucleotide Surveillance Database (RubeNS) is under development. Serology and molecular workshops will be conducted in the WHO regions of the Americas, western Pacific and southeast Asia. The 2019 Global Measles and Rubella Laboratory Network (GMRLN) will take place at the Erasmus Medical Center in Rotterdam. Given that poliovirus elimination is drawing closer, funding for polio laboratories is being wound down. Given that laboratories in the LabNet are intertwined with those carrying out polio surveillance and diagnosis, a strategy to help MR laboratories cope with this change is being developed.

The high incidence rate of measles in 2018 meant that a high volume of samples was tested, with 45 000 samples laboratory tested in the European Region with a 33% confirmation rate. Currently, a large measles outbreak is ongoing in Madagascar, following a period of 10 years with no large outbreaks in the country. Low rates of vaccination, low natural immunity and population malnourishment are leading to a high fatality rate.

Over 5000 MeV sequences have been submitted to MeaNS and just under 400 rubella virus (RuV) sequences were reported to RubeNS. B3, D8 and H1 were the predominant MeV genotypes, while 1E and 2B were the most reported RuV genotypes. Madagascar and Ukraine were the countries with highest incidence rates in the last 12-month period.

Current issues being addressed include kit shortages, timely reporting of sequence data, promotion of the link between laboratory and epidemiological data, and sharing of protocols and samples. The main challenges for the global programme are associated with reagent procurement, shipment of reagents and specimens across borders, annualized accreditation of laboratories, competing priorities, and maintenance of staff competencies.

1.3. Update from RRL Luxembourg

Dr Judith Hübschen (RRL Luxembourg)

Twelve laboratories in the RRL Luxembourg constituency have submitted samples for confirmatory testing so far in 2019. More samples are expected from another nine laboratories. Major discrepancies in the results obtained in the laboratory and at the RRL have been found for four laboratories. In two cases these may have resulted from an error in entering results for a specific sample, while for the other two laboratories, the difference may be due to the samples being weak positive. Only half of the laboratories submitting samples for confirmatory testing submitted the minimum 50 samples recommended by WHO, and two laboratories submitted samples solely for measles testing. The majority of the samples received were DSS, followed by liquid sera and OF.

Comparing the results from confirmatory testing in 2016 and 2019 reveals that the same number of laboratories participating had points deducted in both years, one fewer with concordance issues. While there were no laboratories with more than one issue detected in 2016, there were four in this situation in 2019. Only approximately half of the laboratories obtained a full score. The same observations are valid for rubella confirmatory testing. There has been stagnation in the results, which could be due to changes in test kits or in the results reporting systems.

A new protocol has been established for DSS testing with the Euroimmun recombinant kit. Nine DSS and liquid sera samples were tested in duplicate, both neat and diluted. Good concordance ($r^2 > 0.95$) was observed between liquid and dried spot sera.

The Luxembourg RRL conducted an accreditation visit to the MR NRLs in the Netherlands at the end of 2018 and received an accreditation visit itself on March 2019.

1.4. Update from RRL Berlin

Prof Annette Mankertz (RRL Berlin)

In 2018, the RRL notified 542 cases of measles, 532 of mumps and 58 of rubella, representing an approximate reduction from 2017 of 40% for measles and 20% for mumps and rubella. The rate of laboratory confirmation was similar, at 50% for measles and 8% for mumps. The vast majority of rubella notifications were not confirmed as rubella cases.

The main measles strain circulating in Germany is the genotype B3 Dublin strain. Samples with sequences identical to that of this strain were detected between November 2016 and October 2018, suggesting endemic transmission. However, the analysis of the non-coding region between the matrix and fusion genes (MF-NCR) suggests otherwise. The B3 Dublin strain was first reported in Dublin in February 2016 and has since spread throughout the European Region, leading to many outbreaks, including over 12 000 cases in Romania lasting from 2016 to 2018 and leading to 58 fatalities. In Germany, 168 cases have been detected with this strain, spread throughout the states. The measles strains of the D8 genotype circulating in Germany are mainly of the Gir Somnath variant, but the RRL Berlin does not receive samples from all cases.

The RRL has conducted a pilot study to evaluate the possibility of genotyping serum samples. Twenty-eight sera were tested in parallel with the corresponding throat swabs. It was found that the swabs contained an average of 1000 more MeV genome copies than the sera. Eleven of the 28 sera samples

were successfully amplified and 7 of these (Ct < 33) were genotyped. This is a promising result that could help in closing molecular surveillance gaps when only serum samples are available.

The RRL Berlin often receives samples from recently vaccinated patients. Since 2016, the MeV VA PCR is used in the laboratory to distinguish between wild type and vaccine strains of the virus. Of the 57 specimens tested so far, 14 have been positive for vaccinated strains, 13 of which confirmed by sequencing, and 40 cases were negative and confirmed as wild type when genotyping. Reassuringly, no false positive tests have been encountered.

The countries in the RRL Berlin's constituency have done well with the measles and rubella proficiency test (PT) panels but are not submitting sufficient samples for retesting: only five samples have been received since January. The collection of epidemiological data is strongly hindered by the Data Protection Act in Germany.

1.5. Update from RRL Moscow

Dr Marina Naumova (RRL Moscow)

The RRL Moscow supervises 9 NRLs and 11 SNLs, 10 of which in the Russian Federation and one in Kyrgyzstan. All 20 laboratories passed their proficiency testing in 2018 with full concordance of results for rubella. 19 of the 20 laboratories obtained full result agreement for measles. The kits predominantly used in the RRL's constituency are Vector-Best, Euroimmun and Ekolab. Some NRLs have now switched to Euroimmun from Siemens. When online submission of the proficiency panel testing results was introduced in 2015, 12 laboratories had mistakes associated with the submission of results. This has improved since then and in 2018 only three laboratories committed errors in result submission.

The RRL Moscow conducted confirmatory testing for 956 measles and 848 rubella samples. Of the samples tested for measles IgM, 53.5% were negative, while for rubella IgM samples this fraction was 97.3%. 23.1% of the samples submitted for confirmatory testing were DSS. The procedures for dealing with this type of sample are still being optimized.

Dr Sergey Shulga (RRL Moscow)

The RRL Moscow supervises a subregion consisting of Armenia, Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan, Republic of Moldova, Tajikistan, Turkmenistan and Uzbekistan, in addition to the Russian Federation. There was a significantly increased number of measles cases in this subregion in 2018 compared to previous years. The three countries with most cases were Russian Federation with 2539, Kyrgyzstan with 1008 and Kazakhstan with 576. Within the framework of the measles molecular monitoring, the RRL Moscow genotyped a considerable number of samples from the majority of countries in the subregion, but the volume of testing made it impossible to confirm 80% of the chains of transmission.

Samples collected on FTA® cards are an important part of laboratory confirmation in the subregion and it is apparent that early sample collection is crucial for successful MeV genotyping. Kyrgyzstan did particularly well in this aspect, and genotyping was successfully conducted for all samples submitted. The validation of the FTA® sample processing protocol is underway.

The RRL Moscow has shared new algorithms for the laboratory confirmation of measles, including procedures to deal with IgG and IgG avidity results. The algorithms were well received and all laboratories are eager to adopt them. The RRL continues to participate in accreditation and training activities for the region it supervises, focusing on MR diagnostic methods, genotyping and quality assurance.

An accreditation visit is planned for March 2019 to Azerbaijan; training on measles or rubella PCR detection and genotyping will be conducted for representatives from national laboratories (NLs) in Armenia, Azerbaijan, Kazakhstan and Kyrgyzstan. Visits to several NLs are also planned to provide help in the implementation of molecular testing.

In the questions session that followed the RRL Moscow presentation, issues affecting DSS results were discussed, namely humidity, temperature, virus protective qualities of samples such as blood, source of the filter paper in which the samples were spotted, and how much sample volume was applied.

1.6. Update from GSL London

Dr Kevin Brown (GSL London)

The GSL laboratory at Public Health England (PHE), London, reports to the WHO in the name of the United Kingdom (England, Wales, Scotland and Northern Ireland). It was reorganized in 2018 into a topic-specific structure where laboratory services are commissioned through budgets held by the topic leaders. The structure is still being implemented and has led to changes in key staff line management and responsibilities. The Virus Reference Department of PHE, which includes the London GSL, achieved UKAS accreditation to the ISO standard 15189, a time-consuming process that deviated staff from other activities. The approaching of the deadline for the United Kingdom's exit from the European Union means that contingency plans must be put in place to ensure reagent stocks and essential laboratory services.

The United Kingdom celebrated the 50th year of measles vaccination in 2018. It is estimated that 20 million measles cases and 4500 deaths have been averted in this period. The use of the measles mumps rubella (MMR) vaccine also means that an estimated 1.4 million rubella cases, 1300 congenital rubella syndrome (CRS) cases, 25 000 pregnancy terminations due to rubella infection, 1.8 million mumps cases, and 130 mumps deaths were avoided between 1970 and 2017.

Allegations made against the safety of the MMR vaccine 20 years ago have been discredited and there is currently strong support for the measles vaccination programme. In 2017-2018 91% of 2-year-olds had received the 1st dose of MMR vaccine and 87% had received the two recommended doses by their 5th birthday. It was found that the oldest child in a family is more likely to have received the full vaccination schedule on time than the second or third child due to mounting scheduling challenges faced by parents with a growing family.

Following the elimination of measles from the United Kingdom in 2017, there has been an increase in the number of cases, particularly in London. 1212 cases were reported (995 of which laboratory confirmed) in 2018, up from 759 in 2017. 10% of the cases were imported from other European countries, often with strains sharing the same N450 region sequence being imported multiple times. 50% of patients with measles were over 15 years old. Only two measles genotypes were detected in the United Kingdom in the last year, from different MeV variants.

Following vaccination, individuals mount an effective immune response, but antibody levels may deteriorate with time in some individuals, what is termed a secondary vaccine failure. This leaves them susceptible to the wild virus. Vaccinated persons that become infected with measles do not tend to develop classic measles symptoms and are unlikely to pass the infection on to others. However, the terminology often leads to confusion, especially in the public. The LabNet would probably benefit from a clarification of the terminology and procedures to deal with these cases both in terms of diagnosis and reporting.

Serum samples from measles cases in previously vaccinated individuals tend to be weakly positive or negative for IgM on indirect assays and may be transiently positive in capture assays. They often present a high IgG titre (over 1000 mIU/ml on the Siemens assay), high IgG avidity and very high neutralizing antibody titres (over 40 000 mIU/ml on the third day post-rash). OF samples can also be transiently positive on a capture assay, have a very high test to control ratio (over 20) and are transiently positive by PCR, with a low viral load.

The number of these cases being detected in the United Kingdom is higher than in the past. This is due to various factors: the use of OFs means that the more sensitive capture assays are preferred; PCR is carried out in atypical cases; samples that do not fit the full clinical definition are subject to laboratory investigation; and the improvement in vaccination rates means that more samples from patients who have been fully vaccinated are now tested. Measles in previously vaccinated individuals is considered in cases that present with mild or atypical measles symptoms and that, when tested, show low viral loads and weakly positive IgM responses. These cases are reported, but post-exposure prophylaxis is only offered to immunosuppressed contacts.

Two cases of rubella were reported in the United Kingdom in 2018. One occurred in a pregnant woman (in her 12th week of pregnancy). The RuV strain associated with this case belonged to genotype 2B and was likely imported from Algeria. The baby was carried to full term and did not have congenital rubella syndrome (CRS), but had congenital rubella infection (CRI) and excreted the virus for 5 months. A second case was detected from reflexive testing of all measles-negative samples for rubella and was likely the result of an importation from Ukraine, but it was not possible to determine the genotype for this case.

The United Kingdom's National Measles Guidelines were published in 2017, with a few amendments made since. Guidelines for dealing with measles cases when detected in flight passengers will be published later in 2019. These guidelines constitute an essential resource for local government. Further advice for the protection of children during measles outbreaks and for conducting immunization in schools are under development.

Following the roll out of measles PCR testing to local laboratories, there are concerns with the full reporting of cases to local health authorities. Increased wishes for devolution in Scotland and Wales have meant that insufficient samples are being sent to the London GSL, particularly from Scotland. There are also ongoing concerns with the MicroImmune measles assay, which are yet to be addressed. Finally, clinical supervision of the laboratory testing is unclear at present with the responsibilities of medical staff being altered during the restructuring of the National Infection Service at PHE.

1.7. Update from GSL Atlanta (US CDC)

Dr Paul Rota (GSL Atlanta)

Measles cases increased significantly in the United States in 2018, with 367 cases confirmed in 26 states. 97% of these cases were associated with imported cases. The countries from which most cases were imported were Israel, India and Ukraine. The majority of cases (72%) occurred in unvaccinated individuals and 13% in vaccinated individuals, with the remaining patients having unknown vaccination status. 206 cases have been reported so far in 2019 and outbreaks are still ongoing in New York.

Twenty lineages of measles strains of the D8 genotype were identified, with 69% of the D8 N450 sequences obtained being identical to the MVs/Gir Somnath.IND/42.16 variant. This strain is also present in Venezuela. Further characterization of these cases through the sequencing of the MF-NCR region is ongoing. Measles cases of genotype B3 were mostly associated with the MVs/Kabul.AFG/20.14/3 variant. Although Brooklyn in New York City is a highly populated area, little spread has been seen from the cases reported there.

Serology training updates for the use of EuroImmun and Virion/Serion IgM ELISA kits have been conducted with 74 participants from 59 countries in the WHO African and Eastern Mediterranean regions. During these workshops participants conducted a successful assay, optimized protocols and created calculation sheets.

Session 2 – Serology

Chair: Dr Sabine Santibanez (RRL Berlin)

2.1. Update on kits evaluation and comparison

Dr Mick Mulders (WHO headquarters)

With the discontinuation of Siemens IgM detection kits for MR, it is essential to assess other commercial kits available to respond to the LabNet's needs. MR kits are pre-qualified for WHO procurement, which facilitates acquisition of kits for comparison and later for provision to eligible laboratories.

Currently, potential kits are being evaluated for their sensitivity and specificity at the Canadian RRL. The RRL Berlin has provided well-characterized sera and manufacturers have been approached and are able to supply approximately 66% of the necessary reagents. The test panel contains over 300 sera, including control MR cases, quality control specimens and sera from Brazil, Canada, Germany, India, Indonesia, Mongolia and United States.

An expression of interest will be shared on the WHO website and manufacturers will be invited to apply to participate in the study.

Dr Paul Rota (GSL Atlanta)

Currently, an in-house IgM capture assay is in use in the GSL in Atlanta. However, this assay is cumbersome to maintain, requiring many separate reagents and quality control procedures. It also limits the laboratory's capacity for technology transfer and hence the GSL is interested in switching to a commercial assay.

Kits from Virion/Serion, Euroimmun, Microlmmune, Trinity, IBL, amongst others have been tested using 21 potentially interfering agents, including Parvovirus B19 samples.. As expected, results from weaker samples were found to be more variable between assays.

The GSL aims to collate and analyse the data from any kit comparisons carried out in LabNet laboratories and encourages interested laboratories to contact them with expressions of interest. The data should be anonymised and include the results as well as some of the clinical information. The outcome of this metadata compilation will be shared with the LabNet.

Dr Kevin Brown (GSL London)

The GSL London has been collaborating with VIDRL in the collection of proficiency panel data every year. To assess the kits currently in use across laboratories of the network, the kit cut-offs were normalized and a discriminatory index (DI) was defined as the ratio between the average positive and negative result values.

All measles assays perform well, with high DIs. In 2018, all laboratories passed their proficiency testing and the number of laboratories employing the Siemens kits is decreasing as expected. If laboratories need to discontinue the use of Siemens kits before the kit comparison data is available, any of the other assays in use should perform well.

For rubella, the number of different kits in use is larger and they yield a wider range of results. Capture EIA assays did not necessarily perform better than indirect assays.

Caveats in the interpretation of these data include the fact that the samples were selected for PT rather than assay assessment, this measurement does not measure sensitivity of specificity, and the results are agnostic to the performance of the laboratories using the kit and to the type of assay.

2.2. Serosurveys

Dr Annette Mankertz

The experience and difficulties of conducting children's serosurveys in Germany were presented. Germany conducted two measles, mumps and rubella serosurveys, KIGGS 0 and KIGGS 2. KIGGS 0 tested 13 000 sera from children 2-17 years of age with Siemens kits for measles IgG and Euroimmun kits for rubella and mumps IgG. KIGGS 2 explored 7000 sera for MMR IgG with the same reagents. The comparison of laboratory results was not possible because of the lack of standardization and the need to re-evaluate correction factor on a regular basis. The group was solicited for advice on how to interpret results and the summary is provided in the recommendations section.

Group discussion

Participants discussed the usefulness of IgG avidity testing in an outbreak context, using the example of Ukraine. As vaccine coverage increases across the Region, there is a significant number of measles cases among adolescents and adults who have had 2 documented doses of MMR. Although this is an expected consequence of increasing vaccine coverage, some health authorities are also questioning the role of inadequate records or vaccine failure.

The group discussed the specific situation of Ukraine, where a high number of 2-dose vaccines among the older cases has been reported. Participants debated the usefulness of performing measles IgG avidity testing as a tool to help characterize the outbreak and distinguish between primary and secondary vaccine failure. Knowing there was a national serologic survey performed in 2017 in Ukraine, the consensus was to review those previous data as a first step to assess the need for an IgG avidity study and if confirmed to optimize its design.

2.3. MV neutralizing capacity of two chimeric (human/mouse) monoclonal antibodies

Dr Sabine Santibanez (RRL Berlin)

The European Region still experiences large measles outbreaks. Two of the recent outbreaks with highest number of cases occurred between 2016 and 2018 in Romania and in Ukraine, with over 12 000 and 58 000 cases reported, respectively. The mortality rates associated with these outbreaks were higher than normally expected in the Region.

The profile of incidence rates relative to age varies between countries and outbreaks, but incidence rate is often higher in children and young adults. The level of maternally transmitted antibodies is gradually decreasing due to lower levels of measles circulation, sometimes requiring the vaccination of six-month-old babies during outbreaks.

Vaccination after contact with measles cases is counter-indicated in babies younger than six months, susceptible pregnant women and immunocompromised patients. In these cases, the German Standing Committee on Vaccination (STIKO) recommends immunoglobulin prophylaxis. However, the concentration of MeV-specific antibodies is now lower in plasma donors than it used to be in endemic situation due to less frequent immunity boosting from contact with measles cases. This means there is an increasing need for re-evaluation of previous data on MeV-neutralizing capacity in alternative IgG products.

There is a 3-fold difference between the lowest and highest neutralizing capacity of IgG products for inter-venous application (IGIV) on the German market. Overall, no significant difference in mean is observed between IGIV products and those developed against low-passage Edmonston and D8 strains. The STIKO recommends administering a single dose of IGIV at 400 mg IG/kg of body weight, but no products enriched in MeV-specific antibodies are available on the market. Furthermore, the administration of high volumes of IgG is not comfortable for the patient, the amount of MeV-specific antibodies in an IGIV dose is variable and there is a limited supply of IGIV products, which are also needed for prophylaxis against other infectious diseases.

An alternative approach was explored at the RRL Berlin with the production of monoclonal anti-measles antibodies. The antibodies produced were screened for their MeV neutralizing activity and antibodies from two mice were used to produce seven hybridoma cell lines. The use of immunoprecipitation showed that the secreted monoclonal antibodies (mAbs) bind to the H protein of the Edmonston Zagreb vaccine strain.

Binding studies carried out with the PepStar™ Peptide Microarray identified that mAb RKI-MV-34c binds to the overlapping peptides H₃₀₁GEDSITIPYQGSGK, S₃₀₅ITIPYQGSGKGVSF and P₃₀₉YQGSGKGVSFQLVK, while no linear sequence was identified for mAb RKI-MV-12b, suggesting that this antibody could be binding a conformational epitope. The mAbs RKI-MV-12b and RKI-MV-34c efficiently neutralized all MeV variants and were thus selected for the generation of recombinant chimeric mouse/human antibodies (cmAbs). The latter were found to have neutralizing capacities comparable to that of the mAbs.

The next steps will be to compare these cmAbs with commercial IGIV products, to calculate the MeV-neutralizing capacity in Nectin-4-expressing cells (Calu-3) and to investigate whether the mAb sequence data are helpful in the identification of MeV-Ab sequences in human lymphocytes. If all tests are successful, these cmAbs could be used as IGIV alternatives.

Session 3 – Molecular testing and genotyping

Chair: Dr Sergey Shulga (RRL Moscow)

3.1. MeaNS and RubeNS update

Dr Kevin Brown (GSL London)

The number of sample records in MeaNS has almost doubled since 2016, standing at 46 669 records (47 658 viral sequences) in March 2019. There is considerably less data for rubella, with only 3001 sequences in RubeNS, roughly 6% of those in MeaNS.

There was a drop off in the number of sequences submitted to MeaNS by laboratories of the European Region in 2016-2017. However, subsequent outbreaks across the Region are now reflected in the increase in the number of sequences reported in 2018 and 2019. Sequences appear to be submitted earlier in the year, possibly as countries prepare for the submission of their annual status update (ASU) reports.

The dominant MeV genotypes worldwide are D8 and B3. No MeV genotype D4 sequences have been submitted to MeaNS since May 2018, when there were reports of cases in Portugal and Spain. No H1 sequences have been submitted so far in 2019, following a sharp decrease in the number of sequences of this genotype reported since 2010. This shows that elimination efforts in China (where the sequence was once prominent) have been effective and may lead to the elimination of this genotype. There are now few variants of genotype B3 being detected, with few sequences being submitted of the Dublin and Harare strains. The predominant genotypes reported for RuV continue to be 1E and 2B.

The MeaNS and RubeNS databases are invaluable tools for monitoring virus transmission and documenting importation of cases and countries' endemic status. They are repositories for vast amounts of data and can be used to monitor the elimination of MeV genotypes. However, the data are heavily biased due to inequalities in sample submission between countries, particularly so for

RubeNS. These databases are becoming increasingly important in informing the strategy of the WHO MR programme.

However, there are issues with data consistency and duplication, database performance and security, and the constant need for manual curation. A second version of MeaNS and RubeNS is under development, addressing the current issues and future proofing. The new version will ensure that LabNet needs are being met, that whole genome sequences (WGS) can be handled, and that the website is more responsive, accessible, and can process bulk submissions in a more efficient manner. Training and mEQA proficiency testing modes will be seamlessly integrated in the website. The login privileges will reflect the structure of the LabNet and facilitate access of coordinators to their region's data.

The core functionality and visual layout will remain identical to those of the current version, eliminating the need for retraining users. The initial focus is on achieving a well-curated nucleotide sequence collection system, not a broad sequence analysis or epidemiology platform, although options to provide interoperability with other software tools will be integrated in the website. Feedback from the steering committee and testers can be incorporated into the design during development and testing.

The website and database design are being completed. The data will then need to be curated and transferred across to the new version.

3.2. Molecular epidemiology in the European Region

Dr Judith Hübschen (RRL Luxembourg)

Four imported cases were detected in Luxembourg in 2018. One of these was an unvaccinated 48-year-old male with a history of travel to the Philippines, Switzerland, Thailand, and Turkey. He was infected with a MeV D8 strain. A second patient, unvaccinated, 44 years old, returned from Madagascar with a MeV B3 variant. The sequence obtained was identical to that of cases reported later in Mayotte, France. The final two cases were a 29-year-old mother with her 1-year-old child infected with a MeV of genotype B3, Dublin variant. The mother had unknown vaccination history and the child had not been vaccinated.

Dr Sergey Shulga (RRL Moscow)

Elimination of endemic circulation of MeV genotype D6 was achieved in 2007 in the subregion covered by RRL Moscow (see 1.5 above). The number of measles cases increased again in 2013 due to multiple importations of the virus that led to a large number of genetic variants, including the MeV D8 Republic of Komi strain that became endemic. This was again interrupted in 2015. Since 2016, multiple importations of the virus have been detected which, unlike previously, now align with the strains reported throughout the European Region.

More measles cases were notified in 2018 in the Russian Federation than in any previous year in the past decade. 2539 cases were reported in 2018, up from 721 in 2017. This is a significant increase from the incidence rate of 4.9 to 17.3 cases per million population observed in 2017 and 2018, respectively. In 2017 the majority of cases occurred among unvaccinated individuals (69.7%). 22 cases were imported from 12 different countries and led to several outbreaks of varying sizes, including one with hundreds of cases in Moscow associated with the MeV B3 Dublin variant.

The D8 Frankfurt variant and its descendants have caused multiple local outbreaks for over 12 months. Three genotypes and 15 genetic variants were detected in 2017, 11 of which were detected for the first time in the Russian Federation, mostly as a result of importations. Some transmission chains were short, such as those caused by the H1 Shandong strain, others were long and associated with a higher number of cases, like those caused by D8 Hulu Langat and Cambridge variants. Finally, the D8 Frankfurt variant has been associated with a low-level but sustained transmission in the country.

Genotypes B3 and D8 and 24 genetic variants were detected in 2018, 18 of which were detected for the first time in the Russian Federation, mostly as a result of importations. During 2018 ongoing transmission of D8 Frankfurt variant and its descendants was observed, although the number of cases linked to this transmission was limited. The majority of outbreaks and cases reported were linked to B3 Dublin variant transmission mainly in Moscow and Moscow region, but also in dozens of the regions due to spread of the virus over the country. Finally, these two genetic variants D8 Frankfurt and B3 Dublin were confirmed to be endemic in the Russian Federation with duration of the local transmission for more than 12 months. One more genetic variant D8 MVs/Gir Somnath, which was presumably imported in the beginning of 2018, also demonstrated long-term transmission (Feb – Dec) mainly in Moscow. Other genetic variants of the virus were not characterized by long-term or wide circulation, which reflected their probable rare importation.

During 2016–2018 most countries of the newly independent states (NIS) region reported many measles outbreaks of different scales with the highest incidence level in 2018. All outbreaks according to genotyping data were linked to importation of the different genetic variants of D8 genotype and much rarely H1 genotype. Since the second part of 2017, genetic variant B3 Dublin has become predominant in several countries of the subregion. Unlike before 2016, the NIS subregion in terms of spectrum of circulating measles viruses currently is much like the WHO European Region on the whole.

The laboratories in the RRL Moscow area are performing well and producing high-quality data for the verification of elimination. The timing of sample collection and shipment to the RRL are critical in ensuring the quality of testing. New algorithms for laboratory testing, including IgG and avidity testing are being explored and laboratories are keen on adopting them. The RRL Moscow is also validating a protocol for samples submitted in FTA® paper, participating in training, assuring quality of the testing carried out in its region, and ensuring the collection of genotyping data.

3.3. Experiences using the M-F region: MF-NCR sequencing along a transmission chain defined by contact tracing - first/preliminary data

Dr Sabine Santibanez (RRL Berlin)

The fact that the circulating MeV strains are less diverse means that it is hard to state with certainty whether or not different samples are derived from the same virus. Estimates indicate that only 3% of cases are genotyped, representing a very small proportion of the whole viral population. However, this 3% should be a representative sample and could be used to make estimations of the whole.

The fact that some MeV strains are prevalently reported in Europe may suggest that they originate in the Region. However, the good surveillance systems in place across the Region and high volume of international travel to the Region could also mean that viruses circulating elsewhere may only be detected and described when they are imported into the Region.

The use of extended and whole genome sequencing for MeV is being investigated in several laboratories as a source of complementary information to the classical N450 genotyping window. In Germany, comprehensive epidemiological information is available to support the genotyping data. The RRL Berlin is investigating the use of the non-coding region between the matrix and fusion protein genes (MF-NCR) to distinguish transmission chains.

A local transmission chain spanning the period between March and June 2018 was identified through genotyping and contact tracing. 34 of the 35 cases in this chain of transmission were genotyped and share the N450 sequence of the MeV Gir Somnath D8 strain. With the collaboration of local public health offices, 8 generations of transmission were recorded. Many importations of this strain into Germany occurred from June 2018 onwards from countries in both Europe and Asia, leading to multiple chains of transmission throughout the second half of the year.

The MF-NCR sequence was obtained by Sanger sequencing for approximately half of the cases both in the local transmission chain and in other regions of the country, but sequence could not be obtained for the index case. Low variability was observed in the MF-NCR region within the local chain of transmission. Other methods, including shotgun sequencing, may be attempted to complete the sequence collection for this set of samples and epidemiological data are pending for additional samples.

3.4. Molecular EQA: 4th round results / progress and challenges of laboratories in the European Region: 2018

Dr Paul Rota (GSL Atlanta)

The number of mEQA panels produced for the WHO proficiency testing went up from 49 in 2017 to 86 in 2018 as the European Region is now part of the Global mEQA programme. Some shipments are still pending to Brazil and Indonesia, and the shipment to Oman was delayed due to distribution issues. The report form has been updated, now requesting information on kit expiration dates, use of reference genes and extraction controls. Following complaints that the tubes used in 2017 did not fit standard centrifuge rotors, the Wisconsin State Laboratory for Hygiene (WSLH) is now using standard tubes. Preliminary stability tests indicated that the panels are stable for two weeks at room temperature, but not at 37°C. As in the past, the shipment was done on frozen ice packs and laboratories were advised to store the panel at -20°C.

The panel insert recommends a modified QiaAmp Viral RNA Mini kit procedure. However, many laboratories in the European Region use automated extraction platforms. Following some tests at the US CDC, an elution method that allows for automated sample extraction was recommended, which has also been recommended for similar panels shipped by Instand.

So far, results have been received from 72 of 91 participants globally in the measles mEQA, including 10 retests and no failures. 70 laboratories and 5 of those doing retests have been sent feedback. Of the 88 laboratories participating in the rubella mEQA, 69 have submitted their results, 9 have conducted retests and one laboratory failed. Feedback has been given to 65 laboratories and 8 retest results. All measles retests were on the genotyping section of the test. For rubella, there were two detection and five genotyping retests. The turn-around time was poor, with participants reporting results over 30 days after reception of the panel. Feedback was sent in under 8 days on average.

Dr Myriam Ben Mamou (WHO Regional Office for Europe)

On the 4th round of the WHO mEQA exercise, the European Region switched to the panels provided by the US CDC. 38 of 40 laboratories participated in the scheme (two withdrew). Panels distribution was coordinated between WSLH, RRLs GSL and the Regional Office. Of the 37 sets of results evaluated so far, 34 have passed the proficiency test and 3 are pending.

The fraction of laboratories successfully passing all components of the PT has been steadily increasing for the mEQA schemes since they started in 2015, thanks to tailored training for laboratories facing issues or failing the mEQA. The number of participating laboratories has been increasing, with some laboratories joining, and several showing an interest in participating in future mEQAs.

Session 4 – Regional verification process

Chair: Dr Kevin Brown (GSL London)

4.1. Update on the regional RVC process

Dr Irja Davidkin (RVC)

The RVC was established in 2011 and evaluates the status of MR elimination in each country in the European Region yearly. It reviews the ASU reports and provides feedback to the countries.

The 7th meeting of the RVC took place at the Institute Louis Pasteur in Paris 13-15 June 2018, when for the first time the RVC was able to review the ASU reports received from all 53 countries of the Region. Some reports were again received past the deadline. The quality of the reports in general has been improving, although information required to assess the sensitivity of surveillance systems was still inadequate or lacking in several reports.

The ASU form template has been revised over the years, seeking to improve the quality, consistency and display of the information required for the verification process. The ASU forms for 2017 reflected several changes; for example all laboratory performance information is now grouped in one section of the form, summary tables are included for measles and rubella cases, a single table summarizes molecular epidemiology data for outbreak and sporadic cases, a new type of laboratory profile was created, and tools for visualization of chains of transmission are now supported.

The timeliness and completeness of reporting, rates of laboratory investigations, of discarded cases and of viral detection, and the description of the origin of infection are the main surveillance indicators taken in consideration when awarding elimination status. By the end of 2016, 62% of countries had interrupted or eliminated measles and rubella endemic transmission, while 17% and 26% remained endemic for measles and rubella, respectively. The remaining countries had interrupted endemic transmission for periods shorter than the three years required for the attribution of elimination status. By the end of 2017 the percentage of countries that had interrupted or eliminated measles and rubella endemic transmission was up to 70%, but the percentage that were endemic for measles also increased to 19%. The percentage of countries endemic for rubella transmission decreased further to 21%.

New ways of visualizing number of cases, confirmed cases and characterized cases are being developed to facilitate the work of the RVC and support communication efforts. The NRLs should oversee SNLs, coordinate national MR EQA programmes, and facilitate the access of laboratories to reputable international MR EQA systems. The performance and sensitivity of surveillance systems need to be strengthened and better documented. This would include improvements in the reporting of suspected cases, a higher rate of laboratory investigation of cases, as well as the achievement of higher rates of discarded cases. National procedures for the reporting of MR cases are yet to be implemented in some countries.

The collection of genotyping data is crucial for the verification process to facilitate discrimination between endemic, import-related and sporadic cases. Although most countries now report their sequence data through MeaNS, rubella sequence data in RubeNS still lags behind.

Dr Myriam Ben Mamou (WHO Regional Office for Europe)

Most countries now include good-quality laboratory data in their ASU reports. However, some still need help to better analyse and display their data. Four countries in the European Region are using only RT-PCR, no serology, for the diagnosis of measles, which is contrary to WHO guidelines. A new category has been created of nationally accredited laboratories (NALs) for those laboratories that do not belong to the MR LabNet, but work to a standard approved by the NRLs. Nine countries in the European Region rely on NALs for a large proportion of their data.

Following this presentation highlighting the main verification issues and challenges in the Region from the point of view of the LabNet, the meeting participants discussed how these should be addressed. The outcomes of this discussion are summarized in the form of recommendations in the final section of this report.

4.2. Using sequences to assess the likelihood of transmission

(GSL London)

Increasingly, the laboratories in the LabNet rely on sequence information to assess whether cases are within the same transmission chains. These decisions affect vaccination policies and can determine whether or not a country can prove its elimination status. The tools used so far include complex approaches such as Bayesian Evolutionary Analysis by Sampling Trees (BEAST), which require high levels of expertise for setup and interpretation, and require knowledge, time and computational resources not available to all laboratories.

An approach that allows the estimation of the likelihood that two samples collected within a known interval of time are in the same chain of transmission given the genetic distance between them is being evaluated at the GSL in London.

Substitutions occur in a stochastic manner, not at a defined fixed rate. As such, there is a range of expected substitutions observed at different regions of the genome within a defined time frame. This means that excluding or including samples in transmission chains based on the distance between their sequences is probability based, and not an absolute process.

The GSL London will be developing a plugin for the new version of MeaNS and RubeNS that allows for the plotting of phylogenetic trees along with the number of cases detected for a given sequence

throughout time and, when provided by the user, the total number of cases reported. This would facilitate the production of reports for the ASU and the visualization of transmission chains at the local, regional and global levels.

4.3. Experience and challenges from other WHO regions

Professor Claude Muller (Eastern Mediterranean RVC)

The RVC of the Eastern Mediterranean Region was formed in 2018. This is a heterogeneous region in terms of the resources each country has available. Initial training of NVC members was carried out in a workshop in Tunis in June 2018, attended by representatives of Bahrain, Egypt, Iran, Jordan, Libya, Morocco, Oman, Palestine, Saudi Arabia, Tunisia, Qatar and United Arab Emirates (UAE). The participants were given an overview of the situation in the Region and of the verification process. Training is an important activity of the RVC, as it must ensure consistency and good understanding of the process across the countries. To facilitate assessment, all information will be requested in the form of summary tables or plots.

Dr Paul Rota (Western Pacific Region RVC)

The RVC of the Western Pacific Region was established 8 years ago. The Region includes many countries, with highly diverse population sizes. The Region is doing well in terms of elimination, although vaccination rates are variable across countries. Small countries are given some flexibility in the application of the guidelines.

In addition to presentations, the group also discussed the regional implications of WHO global revised guidance on MR verification and the issue of RT-PCR-only testing strategy adopted in some countries of the Region.

Session 5 – Planning 2019 activities and recommendations

Chair: Professor Annette Mankertz (RRL Berlin)

5.1. eLearning course development update

Dr Myriam Ben Mamou (WHO Regional Office for Europe)

UNICEF's Agora platform has been selected for the eLearning course on recommendation of the WHO eLearning Committee. Its interface can be translated to many languages through Google translation plug-in. The content will be initially developed in English and Russian. Four modules will take priority. The first one will introduce the LabNet and the WHO laboratory manual. The second focuses on EIA and the implementation of IQC (preparation, use and interpretation). Genotyping and sequence data management will be addressed in the third module. The fourth module will focus on the interpretation of laboratory results for case classification.

The next steps include the finalization of storyboards for all modules and the production of the modules' prototypes. The preliminary template is now available and the final e-Learning package is expected to be released in the coming year.

5.2. Follow-up on annexes to include in WHO laboratory manual

Dr Paul Rota (GSL Atlanta) / Dr Mick Mulders (WHO headquarters)

The revised version of the WHO laboratory manual has been launched. It seeks to include the new technologies available to the LabNet and comprises protocols relevant to the various areas of the programme in its annexes:

- Group A: specimen collection, processing, and transport
- Group B: case confirmation, including RT-PCR
- Group C: genetic analysis
- Group D: laboratory quality assessment or management
- Group E: additional characterization of specimens and cases
- Group F: measurement of population immunity

Laboratories have been invited to share their protocols. Those that specialized in specific assays have been approached to provide their methods. The job aids and worksheets included in the manual will be versioned, and protocols will be included for some methods that are not recommended by WHO, with their limitations highlighted.

3. Recommendations

The meeting participants agreed on the recommendations below following discussions throughout the meeting:

Accreditation

1. The accreditation checklist has been revised in order to harmonize and clarify the criteria used across the document. Laboratories are advised that documentation and record control are now part of the checklist. While the minimum score for NRLs/SNLs accreditation is 80%, the score required for GSLs/RRLs is 90%. Labs are requested to fill out both parts 1 and 2 (for sections 1, 2 and other sections as applicable), but assessors will complete the scoring.
2. Laboratories are required to submit the testing algorithms they use for measles, and for rubella with the accreditation checklist as part of Section 1 (the Regional Office to provide instructions for next accreditation round).
3. Laboratories need to be reminded that passing serology (all laboratories) and molecular EQA (if applicable) is mandatory for laboratory accreditation. Partial or provisory accreditation may be given if other criteria are missed, but not following a fail score in PT.

Programme

4. As vaccination rates improve, a proportional increase of cases in fully vaccinated individuals becomes more likely, and waning immunity will need to be considered in the diagnosis. In order to prevent misinterpretation by the public, health care workers, media and epidemiologists, LabNet recommends that WHO and the Strategic Advisory Group of Experts on Immunization (SAGE) provide a suitable terminology for these cases.
5. Guidelines are needed on how to address measles cases in previously vaccinated individuals in terms of diagnosis, classification, reporting, and public health management (SAGE to advise).
6. WHO headquarters should ensure that there is consistency across WHO guidelines (laboratory manual / surveillance guidelines).
7. Funds will be allocated by the US CDC to the IRR to replace WHO procurement of measles and rubella reagents. Network laboratories should register to IRR this year to be able to start ordering supplies (instructions to come from GSL Atlanta).

Serology

8. Laboratories should provide feedback to RLCs / GLC on specific issues with the submission of PT panel results. This will be passed on to VIDRL.
9. An instruction document is needed for submission of PT results to the VIDLR website in multiple languages of the European Region. (VIDRL to develop the document and the Regional Office to translate into Russian).
10. Some laboratories still fail to include in-house controls in their assays. The Regional Office and RRLs will liaise with laboratories in order to understand the reasons behind this (Regional Office to distribute a survey and compile all failures in recent PT and retesting rounds) and provide information on in-house controls commercial sources if needed.
11. For laboratories where kits and reagents are provided by the Regional Office and which cannot obtain suitable in-house controls, the Regional Office will consider the feasibility of supplying positive controls
12. Laboratories should be reminded to send samples to GSL/RRLs for IgM retesting. These samples should be representative of results (positive, equivocal, negative) and originate from measles / rubella suspected cases.
13. In the context of the transition to Euroimmun (IgM) a clear protocol for the calculation and analysis of test results is needed as it differs from that of Siemens kits. The RRL Luxembourg will share the Excel sheet they designed for automatically calculating the ratios and attributing the qualitative results and the Regional Office will distribute it across the region.
14. RRLs and GSL to develop a protocol for DSS that is reproducible in the different contexts of the Region, for the different kits including Euroimmun.
15. The US CDC is compiling previous kit-comparison study data. All laboratories with this type of data (any comparative data) should reach out to CDC with expression of interest by the end of March. The name of the kits tested, a sample identifier (excluding personal identifiable information) and the raw data will be required for the analyses. The Regional lab coordinator to send an email to network laboratories to invite them.
16. RRL Luxembourg is currently evaluating inter-batch consistency of measles IgM NP Euroimmun kits. The GSL/RRLs will gather batch information to circulate across the Region and discuss with the supplier in case of need.
17. Laboratories and WHO coordinators should be aware that studies, such as serosurveys, carried out at different times may yield results that are not directly comparable due to changes in standards and kit modifications. Mixture-modelling and alternative cut-offs should be employed to compare studies. WHO headquarters to share the link to the new serosurveys guidelines when published.
18. Testing serum samples obtained from cases during recent, large measles outbreaks for measles IgG avidity may help to distinguish between primary and secondary vaccine failure. Ancillary surveys should be exploited to inform the need for testing samples.
19. The difference between referral and confirmatory testing (and the related timelines) should be repeatedly explained to the NRLs.

Molecular testing and genotyping

20. Laboratories are reminded that they should be reporting measles and rubella sequences to MeaNS and RubeNS respectively, and that this should be done within 8 weeks of specimen reception to comply with accreditation criteria. They are nevertheless encouraged to submit all sequences even if late.
21. Measles genotype A strains should not be submitted to MeaNS unless there is evidence of direct transmission.
22. A new scoring system is being developed for the mEQA scheme. This would weigh results so that obtaining the incorrect results would lead to a fail, while less serious errors would be

weighed less heavily. RRLs, GSL, GLC and RLCs should review the new scoring system when shared by CDC before the Global Measles and Rubella Laboratory Network meeting.

23. Fast Track Diagnostics rash and fever assay has been shown to miss specific measles strains, which has been published in J Clin Microbiol. 2019; 26;57. This assay should not be used in the network until the issue is solved. Three laboratories in the Region use a monoplex assay of the company and should be aware of this issue.

Regional verification process

24. Given that countries have different case definitions, NVCs are reminded to provide respective definitions in the ASU to be considered by the RVC.
25. Currently the form submitted to the RVC requests that the countries indicate the testing strategy used. However this is not specific for measles or rubella, and the algorithms for these may be substantially different. The LabNet recommends that full algorithm flowcharts used for measles and rubella are attached to the form (by the WHO secretariat). This would help ensure the algorithms are appropriate in the country setting. When deemed inadequate, the surveillance system of the country would be considered insufficient.
26. It is the laboratories' responsibility to interpret laboratory data, not the NVCs'. NVCs should fully engage the laboratories in the process of ASU preparation. Discarding cases solely on the basis of negative PCR results is not adequate as indicated in the laboratory manual (https://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual_section6.7/en/).
27. A high-quality measles surveillance system is not indicative of good rubella surveillance. A high-quality fever/rash surveillance system can be. In the absence of national case-based rubella surveillance, only laboratory-based surveillance of rubella integrated with a high-quality fever/rash surveillance system is acceptable to verify rubella elimination (WER 2018; 93: 544-552).

Training

28. Based on serology and molecular EQA results, RLC, RRLs and GSL to decide on the training needs and activities for 2019, to be addressed either by webinar or workshop with selected participants. RLC to follow-up with the RRLs and GSL.
29. RRLs and GSL are invited to share protocols for the laboratory manual annexes (keeping in mind these will be public on the WHO website).

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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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