

**WHO EUROPEAN REGIONAL
MEASLES/RUBELLA LABNET MEETING
FOR WESTERN AND CENTRAL EUROPEAN COUNTRIES,
TURKEY AND GEORGIA**

27-29 JUNE 2016

BUDVA, MONTENEGRO



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ACCREDITATION

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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
ECDC	The European Centre for Disease Prevention and Control
EEA	European Economic Area
ELISA	Enzyme linked immunosorbent assay
EMRO	World Health Organization Regional Office for Eastern Mediterranean
ES	enhanced (active) surveillance
EQA	External Quality Assessment
EVAP	European Vaccine Action Plan
EU	European Union
EUR	European region
EURO	European regional office
GSL	Global specialized laboratory
HH6	Human herpesvirus type 6
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LabNet	Laboratory network
MCV	Measles-containing vaccine
MeaNS	Measles Nucleotide Surveillance
MeV	Measles virus
MR	Measles/Rubella
MRLDMS	Measles and rubella laboratory data management system
NGS	Next Generation Sequencing
NIS	Newly independent states
NL	National laboratory
NRL	National reference laboratory
NVC	National vaccine committee
OF	Oral fluids
PAHO	Pan-American Health Organization
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
RVC	Regional Verification Commission

SAGE	The Strategic Advisory Group of Experts on Immunization
SIA	Supplemental immunization activity
Tessy	The European Surveillance System
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America

Executive summary

The 2016 meeting of the WHO western and central Europe, Georgia and Turkey Measles/Rubella LabNet took place in Budva, Montenegro, from the 27th to the 29th of June.

Participants of the meeting were:

- Representatives of the Measles/Rubella laboratories of the following countries: Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Israel, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, the former Yugoslav Republic of Macedonia, Turkey and United Kingdom;
- Observers from the Department of Microbiology of the Institute of Public Health in Pristina, and from the Center for Disease Control and Prevention of the Institute of Public Health in Podgorica;
- Representing Instand e.V.: Dr Oliver Donoso Mantke;
- Rapporteur: Dr Ana Penedos from the Virus Reference Department of Public Health England in Colindale;
- Consultants: Dr Vicki Stambos from the Victorian Infectious Diseases Reference Laboratory of the Doherty Institute and Dr David Featherstone;
- Centers for Disease Control and Prevention: Dr Nino Khetsuriani from the CDC South Caucasus Office;
- Regional Verification Commission: Dr Günter Pfaff (Chair)
- World Health Organization: Dr Mick Mulders, Global Coordinator of the Global Measles Rubella LabNet, Dr Myriam Ben Mamou, Regional Coordinator of Vaccine-preventable Diseases and Immunization, Ms Mina Brajovic, Head of the WHO Country Office Montenegro, Dr Patrick O'Connor, Team Lead of Vaccine-preventable Diseases and Immunization, Dr Sergei Deshevoi, Medical Officer of Vaccine-preventable Diseases and Immunization, Mr Robert Jensen, Programme Assistant of Vaccine-preventable Diseases and Immunization and Mr Theodoros Kaloumenos, Technical Assistant of Vaccine-preventable Diseases and Immunization.

Presentations and discussions during the meeting sessions allowed to develop operational recommendations in the following areas: surveillance, molecular epidemiology, internal quality control, proficiency testing, molecular EQA, reporting to WHO, verification process, training, resources and communication, and polio containment. The full recommendations are included at the end of this report (Summary and Recommendations section).

1. Introduction

The EUR MR LabNet consists of a group of laboratories accredited by WHO for performing measles and rubella diagnosis, and laboratory case investigation and surveillance. This network of laboratories plays an essential role in informing the WHO and local governments on measles and rubella circulation in the region, providing crucial data necessary for decisions with impact on immunisation programmes and directives for elimination.

Here, we report the presentations and discussions occurring during the meeting of the western and central European, Georgian and Turkish laboratories, which took place in Montenegro from 27th to the 29th of June 2016. A similar meeting for the Russian speaking countries took place in Montenegro from 29th June to 1st July 2016, with a combined day of presentations on 29th June 2016.

2. Sessions of the meeting

Dr Vesna Macic, Montenegro Ministry of Health Representative; Dr Mina Brajovic, Head of the WHO Country Office in Montenegro; Dr Myriam Ben Mamou, WHO Regional Coordinator of Vaccine-preventable Diseases and Immunization

The participants were welcomed to Montenegro by Dr Vesna Macic, who wished them a successful meeting and pleasant stay in Budva.

In Montenegro, measles and rubella endemic transmission was interrupted in 2014 and no endemic transmission has been seen in the last twelve months. Another two years are required to prove elimination of measles and rubella in the country. The main challenges are the low immunisation rates, particularly in children, due to MMR vaccine refusal. Measles, rubella and polio surveillance are receiving special emphasis as Montenegro seeks to improve national surveillance capacity for these diseases.

Dr Mina Brajovic also welcomed the participants to Montenegro and stressed that elimination of measles and rubella is the core goal in WHO's Global Vaccine Action Plan. All member states are engaging with the necessary activities to support elimination, with over half having managed to interrupt endemic transmission, indicating that elimination is possible and that EURO is on the right track to achieve it.

The main vulnerabilities identified that may impede elimination are population changes and the existence of immunity gaps. In order to maintain momentum and sustain current achievements, increased political and technical support is required, especially in the improvement of surveillance and verification of elimination. Laboratory investigation of cases is one of the key pillars for elimination and hence efficient and integrated laboratories are essential for the elimination effort. The role of the LabNet and of the seventy-one accredited laboratories is thus to promote high-quality testing and to coordinate action towards elimination.

The aim of this meeting is to share achievements and challenges in sustained high-quality laboratory monitoring and verification of measles and rubella elimination, and Montenegro hopes to learn from the interactions with the global specialised laboratory (GSL), regional reference laboratories (RRLs) and national reference laboratories (NRLs).

Dr Myriam Ben Mamou asked the participants to give feedback on the new format of the meeting, designed to deliver training, promote discussion and better assess and address issues faced by the laboratories. She welcomed the expertise and thanked the participation of Dr David Featherstone, Dr Vicki Stambos, Dr Oliver Donoso Mantke and Dr Günter Pfaff. She wished the participants a good meeting, with helpful interactions and outcomes.

Session 1A – External quality assurance and Internal quality control

Chair: Prof Annette Mankertz (RRL Berlin)

1A.1. External Quality Assurance for IgM serology

Dr Vicki Stambos (The Doherty Institute, Australia)

The main objectives of the measles and rubella IgM proficiency testing (PT) are to assess proficiency of laboratories in the WHO global network, identify issues with assays in routine use, verify the accuracy of data reporting, check assay validation criteria and measure timeliness of reporting (results reported within 14 days of receiving the proficiency panel).

All serum samples in the panel are pre-issue screened for HIV, HEB, HEC, for measles IgM (using Siemens Enzygnost Anti-measles virus IgM EIA and Biorad Platelia Measles IgM Capture EIA) and for rubella IgM (with Siemens Enzygnost Anti-rubella virus IgM EIA and Nova Tec Novalisa Rubella Virus IgM μ -Capture ELISA). All samples are filtered to minimise contamination, with an aliquot of each sample stored at room temperature and visually checked for contamination on a daily basis. After two weeks, these aliquots are tested using the Siemens kits to verify that there are no significant declines in IgM levels, that positive IgM samples are still above cut-off and no false positives are detected.

Last year, 20 samples composed the panel (01502) that was dispatched to participants, 6 of them were measles IgM positive, 6 rubella IgM positive, 1 IgM positive for parvovirus B19, 1 IgM positive for dengue virus and 6 IgM negative for both measles and rubella. A user-friendly website was created for result submission, where laboratory details are saved for future submissions and which introduces mandatory fields to limit relevant data omission. The website also makes use of drop-down fields to minimise typing/transcription errors and the VIDRL is automatically notified when results are submitted, eliminating problems with incorrect e-mail addresses.

The PT score algorithm has been reviewed to take into account, not only test results, but also result interpretation, timeliness of reporting and completeness of assay and validation data. The scoring now takes into account the OD reported, kit information, use of internal quality controls and in-house ISO 15189 validation. Obtaining the correct result and interpreting it correctly account for 75% of the PT score, with a maximum of 20 points being attainable. Points are deducted for incorrect data, interpretation and inconsistencies between the data and its interpretation.

The remaining 25% of the score are associated with completeness of information and timeliness of reporting. To obtain the highest score, the laboratories should provide complete kit data and validation information, as well as validation criteria and in-house controls within 14 days of receiving the panel. 0.55 points were deducted for each week delay in submitting the results.

In the last year, 237 laboratories participated in the measles PT and 235 in the rubella one. For the EURO region in specific, 69 laboratories participated in both measles and rubella PT exercises, with one laboratory submitting 2 sets of results for measles and another submitting 3 sets of results for rubella.

In EURO, 6 commercial kits were employed in the measles PT panel and 12 in the rubella panel. The kits most used were the Siemens IgM kits for both measles (utilised by 62% of the laboratories) and

rubella (employed by 56% of the laboratories). Few laboratories used in-house assays for measles and rubella IgM detection: 2 and 1 for measles and rubella, respectively.

The vast majority of laboratories submitted sufficient kit information, although fewer submitted the required cut-off value. All laboratories reported the use of positive and negative controls, but only 75% had in-house controls. 3 and 2 sets of results were reported using expired kits for measles and rubella, respectively. Most laboratories submitted the PT panel results within the recommended 14 days.

97% and 90% sets of results submitted obtained a perfect score for the measles and rubella panels, respectively, but only 44% of the submissions achieved the perfect score in the PT panel overall, due to insufficient information provided. 99% and 96% of laboratories obtained a PT panel score over 90% for measles and rubella, respectively.

The majority of EURO laboratories utilised the website to submit PT results, which will be updated in response to user feedback obtained in a survey carried out by VIDRL, addressing reported issues with login and result submission.

Following her presentation, Dr Vicki Stambos demonstrated how to submit PT results using the website and answered questions and concerns of the participants. Slides detailing the login and submission processes for the website were circulated with the meeting materials to the participants and may be requested by anyone interested.

1A.2. External Quality Assurance for molecular detection and sequencing (mEQA)

Dr Oliver Donoso Mantke (Instand e.V.) and Dr Sabine Santibanez (RRL Berlin)

Instand e.V. (Instand) is a non-profit organisation and only one of two institutions providing molecular quality assessment schemes. Instand collaborates with over 80 countries to provide external quality assessment (EQA) screens, being involved in over 350 serology and molecular programs, 68 of which in virology, the most recent in Zika and Chikungunya viruses. Appropriate samples are obtained from scientific partners and sent to laboratories.

Instand was asked to prepare a molecular EQA (mEQA) panel for the WHO EURO laboratories after the 2015 Global Measles and Rubella Laboratory Network meeting and laboratories were invited to take part in the scheme started in November 2015. Although laboratories are expected to support the mEQA exercise costs, the WHO will support laboratories with low resources. Instand's protocols are being expanded to encompass a full set of results, including genotyping and sequencing of measles virus (MeV). CDC and Instand are collaborating to harmonise the respective proficiency testing schemes at the regional and global level.

Of the 37 laboratories invited to participate, 34 registered for the measles mEQA exercise. Samples were shipped to National Laboratories and all laboratories had reported their results by the end of May 2016. The mEQA sample panel included 3 samples with different genotypes and 1 negative sample. The positive samples were selected to match WHO named strains from the B3 (MVi/Harare.ZWE/38.09), D4 (MVs/Manchester.GBR/10.09) and D8 (MVs/Frankfurt Main.DEU/17.11) genotypes, and consisted of lysates of infected cells on FTA disks.

The mEQA consisted of two categories: qualitative genome detection and type identification. All laboratories identified correctly positive and negative samples. However, 2 of the 30 laboratories registered for the type identification section of the test did not report results, one of them because of weak PCR amplification precluding sequencing. All remaining laboratories reported correct results, leading to a 93.3% success rate in the second part of the mEQA.

A new category has been introduced after the first round of the exercise that takes into consideration sequence quality. Its results did not impact on the attribution of certificates. Of the 28 laboratories that submitted type identification results, 27 provided sequence data. 21 laboratories (in 20 countries) sequenced all samples correctly (75% of the laboratories registered for type identification). Errors included incomplete N-450 ends (the most frequent error), single nucleotides missed or added, mismatches or unknown bases ("N"). The majority of laboratories utilised in-house assays. Issues with training and documentation were identified.

There were 30 registered laboratories for the rubella mEQA, all of which had submitted their results by March 2016. The rubella panel consisted of 3 positive and 1 negative sample. Two of the positive samples were replicates of genotype 2B (RVs/Duesseldorf.DEU/35.13) and the third was from genotype 1G (RVi/Prahova region.ROU/25.03). The rubella samples were prepared and shipped in the same manner as the measles ones.

96.7% of the participants identified correctly positive and negative samples and 62.5% completed the type identification correctly. Only 13 of the 19 laboratories that provided sequences had sequenced all samples correctly, a result lower than that obtained by a similar mEQA scheme run by WHO. In-house assays were, again, the most common. In contrast to MeV, sequences were found to have more serious errors (e.g., long stretches of "Ns", nucleotide mismatches at several positions, incomplete ends). While for measles the issues were mostly identified at the interpretation step, for rubella sequencing, there appear to be problems at the laboratory level as well.

Overall, the first Instand mEQA was successful and will form the basis of future mEQA schemes. Information on sequence quality will be included in the assessment in later exercises, with the type identification and sequence errors to be reported on the certificates. The assessment will also be stricter, with the length of sequence required being set at 450 and 739 nucleotides for measles and rubella, respectively. Instand is collaborating with the CDC and the WHO so that sample preparation, quality and shipping is comparable across all regions. Standard sheets for reporting of methods and results are currently used, but an online reporting system that includes sample submission to MeaNS and RubeNS is under development.

After the presentation, the participants were encouraged to provide feedback, ask questions and suggest improvements to the mEQA scheme. The main decisions/conclusions resulting from this discussion are included in the recommendations from this meeting (see Summary and Recommendations section at the end of this report).

1A.3. Internal quality control procedures

Dr David Featherstone (Senior Scientist / Consultant)

Laboratories provide crucial information to the disease program, such as confirmation of suspected cases, differential diagnosis and identification of transmission chains. This information may lead to

decisions that cost millions (e.g., vaccination campaigns) and hence it is imperative that results are timely and accurate, both locally and on a larger scale.

Although no assay is perfect, most assays employed in the measles and rubella LabNet are performing well, despite minor issues with sensitivity and specificity having been reported. When assays are less specific or sensitive, a variety of approaches can be adopted to improve the process of laboratory result-based decision-making. These could include the implementation of confirmatory testing or the test of a specified number of samples prior to decision-making, for example. These methodologies are particularly important in low-incidence regions as the positive predictive value of ELISA IgM tests diminishes.

Given the abundance and variety of factors affecting the results obtained (e.g., sample transport, quality and storage, SOPs, result recording and reporting, personnel), it is essential to have internal quality controls (IQC) in place in order to detect, evaluate, and correct errors due to test system failures, environmental conditions or operators.

The WHO MR accreditation programme monitors laboratory performance within the LabNet by assessing all laboratories on a regular basis under the same set of criteria. An accreditation checklist provides guidance to a series of quality assurance procedures, one of which is the routine use of IQC, which comprises a number of procedures:

- Only validated assays are used for IgM detection;
- Kit validation criteria are followed;
- In-house positive control(s) are used for EIA;
- The monitoring of in-house and kit controls is presented as a graphic display;
- Pipettes and thermometers are regularly calibrated;
- Daily monitoring of temperatures of appropriate equipment;
- Currently certified thermometer available;
- Appropriate controls are used for each PCR run (if performed);
- SOPs are acceptable and readily available;
- A document control system is in place;
- Results are recorded electronically and backed-up.

All controls included in the kit should be used and all validation criteria results should be documented on the assay worksheet. Since kit-supplied controls may vary with time or lot number, and may not be representative of clinical samples, an in-house control should be included in all assays to provide a benchmark for assay variance. In-house controls should be prepared in single-use aliquots for a long period of time (~1 year), be specific for the relevant assay and fall within the mid-range of the assay results. If sample volumes are insufficient, it may be necessary to pool several samples. When a sample is too strongly positive, a negative may be used to dilute it. Where insufficient positive IgM samples are available to use as in-house controls, the Regional Laboratory Coordinator or the Regional Reference Laboratory (RRL) should be contacted. If the IQC result is out of the acceptable range, possible issues/errors should be identified and corrected. Only then should the test be repeated and reported (if successful).

1A.4. Graphical monitoring of controls / Westgard rule

Dr David Featherstone (Senior Scientist / Consultant)

Most measles and rubella ELISA assays provide quantitative assay results (OD value), from which a qualitative result is generated (positive, negative and, in some assays, equivocal or reactive). The quantitative result can be used to monitor the assay, helping to detect abnormal results and identify trends before they become problems. Observing trends and potential issues is facilitated if the results are represented graphically.

For this, a Levey-Jennings chart can be used. This chart represents a series of results for a control sample, overlaid with the mean and standard deviation (SD) of results for the same sample. The mean and SD are established from the first 20 replicates of the control sample. Up to 5 replicates can be tested in each run to establish the mean/SD and if multiple operators run the test, then all should be part of establishing the guiding values. A maximum of 2 outliers may be excluded from the mean calculation and the replicates should fall within 2 standard deviations of the mean. Once the guiding values for the mean and SD have been established for a specific control, if the value of that control obtained in subsequent runs is within ± 3 SD of the mean, it can be accepted as "in-control".

Westgard rules are often applied to interpret Levey-Jennings chart when two or more controls are used, although they can be employed when a single control is utilised as well. By this means, not only excessive deviations from the mean can be detected, but trends can also be observed. Three rules should be considered in the monitoring of assays:

- $1_{3\sigma}$: when one IQC result exceeds the mean by over ± 3 x SD. The run should be rejected in this case;
- $2_{2\sigma}$: when two consecutive IQC results exceed the same mean by over ± 2 x SD. This should be taken as a warning that there may be issues with the assay.
- 10_x : when ten consecutive IQC results fall on one side of the mean. Again, this should be taken as a warning that there may be issues with the assay.

Before looking at Westgard rules, the kit validation criteria must be verified. Westgard rules are only relevant if the run is valid according to these criteria. It is useful to establish standard approaches to test failures, which can be incorporated in the SOP for the assay. Troubleshooting guides, often provided with equipment and reagents, can be followed to find and address issues. Examples of common issues leading to test failures are:

- Degradation of reagents or kits;
- Operator error;
- Failure to follow manufacturer's instructions;
- An out-dated procedure manual;
- Equipment failure;
- Calibration error.

For the IQCs to be representative and informative, it is important that they are adequately prepared and stored appropriately with the remaining ELISA reagents. Addition of diluent to concentrated single-use aliquots can compensate for volume change during storage. Assay records should include the lot number of the IQCs, kit controls and assay, operator name and date of assay. When plotting the values of the IQCs and kit positive controls, the date, mean, 1, 2 and 3 x SD values, and the upper and lower limits of the kit controls should be shown.

In the Q&A following the presentation, some points were added for clarification on the use of Levey-Jennings charts and Westgard rules:

- The mean and SD can be calculated retrospectively if the same IQC has been used.
- When a different lot is started, the same mean and SD should be valid. If not, run 5 replicates in 4 runs to establish a provisory mean and SD and when 20 runs have been carried out, replace the provisory values.
- Westgard can be used to monitor qPCR results, so long as the exponential nature of the assay is taken into account.
- Commercial sera are available to use as IQC (e.g., Optitrol positive controls for measles IgM) and the NIBSC also has positive material for serology and molecular assays.
- The amount of variability expected depends on the IQC and the assay. Differences between operators are expected, but become more concerning if a particular operator obtains very variable results.

Dr David Featherstone then guided the participants through an example data set for the implementation of Westgard testing: calculating the mean, standard deviation and its multiples, obtaining the representative plot and interpreting it. The slides and workshop record will be made available to those seeking training.

Session 2A – Providing quality laboratory verification data (How to prepare laboratory sections of the NVC report)

Chair: Dr Judith Hübschen (RRL Luxembourg)

2A.1. Verifying measles and rubella elimination: laboratory information required in the Annual Status Update (ASU) – template

Dr Patrick O'Connor and Dr Myriam Ben Mamou (WHO regional office for Europe)

The participants were introduced to a new template for the Annual Status Update (ASU) report. It has been found in the previous ASU that the figures submitted for measles and rubella epidemiology were different from the WHO records. Laboratories and epidemiology would benefit from the harmonisation of common definitions employed. The National Verification Committee had issues identifying some laboratories' profile, evidence of proficiency and credentials.

Laboratories must characterise chains of transmission – sequencing is recommended on at least 80% of the chains of transmission. Thus, distinct MeaNS sequence IDs should be submitted as well as the number of chains of transmission that were genotyped. Automated upload of data is enabled in MeaNS, (see below NL listing functionality) facilitating data submission and reducing errors.

The first part of the ASU refers to the laboratory performance, requiring laboratories to submit their approach to measles and rubella disease investigation. Although IgM testing is the gold standard recommended by the WHO, some countries use molecular detection for exclusion of cases. Information on standard testing as well as on testing algorithms and applied definitions should be provided. The type of test carried out will depend on sample type and time of collection: a field is provided to specify the test undertaken and the reason for its selection.

When the number of suspected cases tested is not consistent with the initial tables, laboratories should provide an explanation, such as accounting for private laboratory results for instance. The table dedicated to sporadic cases should not overlap with that destined to list outbreaks. In the former each line should represent a single case, while in the latter each row corresponds to an outbreak.

In order to achieve measles and rubella elimination in a greater number of countries, laboratory data is crucial to identify at-risk populations and cross-border outbreaks. It is therefore very important that all chains of transmission are reported, independently of size. To facilitate the visualisation of transmission events across the EUR, the ambition is to convert the case data submitted into a graphical representation.

2A.2. Using MeaNS and RubeNS to generate sequence data tables for ASU

Dr Kevin Brown (GSL London)

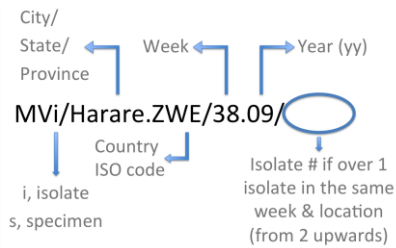
The Measles Nucleotide Surveillance database (MeaNS) can be found at www.who-measles.org. It is a fundamental tool in tracking MeV sequence diversity and to monitor the elimination of strains.

The most diverse regions in the MeV genome are the 450 nucleotides coding for the carboxyl end of the nucleoprotein (N-450) and the non-coding region between the matrix and fusion protein genes (MF-NCR). Given that the sequencing of the MF-NCR is more challenging than that of the N-450, the latter is routinely used for genotyping, often in conjunction the hemagglutinin (H) gene.

MeV strains can be divided into 8 clades (A-H) and 24 genotypes (A, B1-B3, C1-C2, D1-D11, E, F, G1-G3, H1-H2), with A comprising the vaccine strains. The WHO has selected 24 reference strains to represent each measles genotype. However, a number of genotypes has not been detected for many years, being considered eliminated. At present, the majority of cases worldwide belong to one of eight most common genotypes. In order to reflect the evolution of the virus and facilitate communication, common circulating strains become named strains.

Since 2006, tools have been available on MeaNS for genotyping, sequence comparison and identification, weekly reporting to the WHO and upload of sequences to GenBank. A non-registered user can examine new events of measles and the distribution of genotypes. Users registered on the website should be able to upload and analyse sequences. Currently, public and private modes of sequence submission are available, with sequences submitted using the latter made available only to the country submitting the sequences and WHO. Optionally, sequences can be submitted to GenBank as well.

A registered user may submit new sequences through the “Data” tab. The database is dedicated to wild-type circulating virus, not vaccine strains, and hence no sequences belonging to genotype A should be uploaded. The sequences are stored using the WHO name, so it is essential to name strains correctly and consistently before submission as this field cannot be changed after submission. The WHO name should look like:



Alternatively, when the relevant information is input, the database will create the WHO name automatically. The name can be edited prior to submission. When many identical sequences are being submitted, a template can be created, allowing for minor modifications to be carried out before submitting each sequence. If the user wishes to submit the sequence to GenBank too, the information is automatically processed accordingly and submitted to the website. The user will be receive any queries referring to the GenBank submission directly.

Tools to define the genotype, obtain the phylogeny and find matches of named strains for an uploaded sequence are available, and the user may download a list of all strains matching the sequence if desired. Additionally, countries may consult the circulating genotypes by month and WHO region, as well as obtain the sequence summary for the country (by choosing summary, country, period, chart overview). To obtain a country summary for the ASU, tables detailing sequences submitted can be downloaded in comma-separated values (csv) format, using the NL Listing functionality (under Sequence analysis tab of MeaNS frontpage). When strains submitted have become named strains, this information will be updated in the report obtained.

The Rubella Nucleotide Surveillance (RubeNS) database serves the same purpose as MeaNS and can be found at www.who-rubella.org. Unlike in MeaNS, the user of RubeNS has control of password changes and can use wildcards to search for samples. The more limited number of sequences available in RubeNS means that exact matches to uploaded sequences are unlikely or less numerous.

As in MeaNS, tools for genotyping are available on RubeNS, and the method approved by the WHO is described on the website. Phylogenetic analyses of strains show reference and key strains. Again, a tool for line listing for ASU is available and the results can be downloaded.

During the Q&A session following the presentation, Dr Kevin Brown responded to the participants' questions, clarifying several points:

- If desired, multiple sequences can be uploaded simultaneously to MeaNS, using an Excel template, which will be made available.
- If the sequence the user is attempting to submit is not of the correct length for the relevant region, an error message will be displayed and the closest genotype (for N-450 sequence) determined. If the sequences to upload are longer the relevant region, but contain the sequence window of interest, sequences will be automatically trimmed and the correct length submitted.

- Sequences submitted to GenBank that do not contain the information required by the MeaNS database will not be automatically uploaded into the latter.
- All regions of the MeV genome can now be submitted to MeaNS, including any of the genes, the MF-NCR or the whole genome sequence excluding termini (WGS-t).

A demonstration of sequence submission to MeaNS followed. Training materials are available to all interested.

Session 3A – Measles and Rubella Laboratory Data Management System (MRLDMS) 2 training

Chair: Dr Kevin Brown (GSL London)

3A.1. MRLDMS 2 training

Dr Theo Kaloumenos (WHO regional office for Europe)

The participants were introduced to the new MRLDMS2 database. The development phase is now complete and awaiting feedback to go live. Versions will be available in English and Russian. A test instance of the application is accessible online at ldms.euro.who.int:2687. The goal of this database is to provide an automated import tool for laboratory data as well as working as a LIMS system for laboratories where one is not in place already. In this manner, the WHO hopes to address double entry of data and simplify data submission through a bulk upload tool.

The database is highly customisable, allowing laboratories to create own specimens and tests, and to use their in-house developed upload tools. The levels of access to the database are also customisable. Laboratories only have access to their data, while RRLs may see data from subordinate laboratories as well. Several samples from the same case can be linked.

A large variety of fields are included in the application to permit its use as a LIMS system. However, only some of these fields are mandatory. A default value can be defined for a field, fields available in the laboratory data but not defined in the application can be created and the field names used in the laboratory can be matched to those defined in MRLDMS2. If no specimens have been tested, laboratories must report so on a monthly basis, although weekly reporting is recommended. Additional information on a specimen or case can be added after submission (e.g., new test result) as the system recognises when a specimen has been added already and updates the corresponding information.

Session 4 - Global and Regional updates

Chair: Prof Claude Muller (RRL Luxembourg)

Participants were welcomed into the joint session with all the EUR laboratories and reminded of the essential role of the LabNet in the goal of elimination of measles, rubella and CRS in the EUR. Colleagues involved in the global polio elimination initiative were encouraged to share their experiences, as there is much to learn from that programme.

4.1. Update from the Regional Verification Commission

Dr Günter Pfaff (RVC)

Although all six regions of the WHO have set elimination goals for measles and rubella, most (with the exception of AMRO) are behind their target. In the EUR, 21 countries have eliminated measles and 20 have done so for rubella. In order to demonstrate elimination of measles and rubella transmission, countries must prove that endemic transmission has been interrupted for at least three years. This must be done in the context of a robust surveillance system that can detect cases when they occur.

Furthermore, the absence of endemic cases, in a well-functioning surveillance system, must be supported by genotyping evidence, a requisite that is becoming increasingly more important to understand transmission chains in the EUR. The information collected reveals simultaneous but separate outbreaks as well as long outbreaks occurring across countries.

While, due to historic reasons, epidemiology and laboratory often evolved separately, there is now the need to obtain a coherent overall picture by coordinating the information collected by both. The Regional Verification Committee was started in 2012 and met last in October 2015. The 2016 meeting approaches and laboratories are encouraged to keep to the timeline in reporting to the RVC.

In order to prove a high-quality surveillance system, laboratories must demonstrate that all suspected cases are reported and genotype information is obtained to complement the epidemiology so that separate outbreaks can be identified. If the information is incomplete, then the measles and/or rubella elimination status is unknown.

Interruption of endemic transmission can be verified when there is no in-country chains of transmission lasting longer than 12 months in period (for period) of 36 months/3 years. However, if the immunisation level is below the recommended, the country remains at risk. A new category for endemic transmission re-established has been introduced for countries that had eliminated MeV or RuV before. The distinction between import-related (imported strains that have circulated less than 12 months) and endemic transmission must be supported by complete epidemiology and sequence information. This is particularly lacking for rubella.

The EUR of WHO is composed of 53 member states, comprising over 900 million inhabitants and a large cultural and social diversity. The main challenges in each country may vary, but will in general include the achievement of high vaccination coverage, the elimination of immunity gaps, the implementation of a high-quality surveillance system and the improvement of knowledge and training. To obtain a more detailed picture of measles and rubella disease, more information is necessary not only at the country and regional levels, but also at the subnational level. In particular, countries are encouraged to provide the RVC with any outbreak maps they may have produced.

The RVC urges all member states to:

- Support verification activities by providing all needed national and sub-national data, information and documents to their national verification committee (NVC), thereby facilitating timely submission of complete and comprehensive annual status reports;
- Improve the quality of rubella and CRS surveillance and increase the level of laboratory investigation of rubella suspected cases and reporting of rubella sequence data;

- Support capacity-building of the Regional Measles and Rubella Laboratory Network, and improve the capacity to link sequence and surveillance data;
- Ensure that adequate documentation on outbreaks, including supplementary immunisation activities and outcomes, together with adequate outbreak reports are provided to the NVC;
- Consider activities to increase population immunity by improving routine immunization coverage and/or carrying out targeted supplemental immunization activities.

The elimination status of the EUR member states is summarised in the table below:

Elimination Status	Number (%) of Countries in Category	Countries (<i>underlined when disease is eliminated</i>)	
		Measles	Rubella
Interrupted	32 (60%)	<u>Andorra</u> , <u>Armenia</u> , <u>Azerbaijan</u> , <u>Belarus</u> , Bulgaria, Croatia, <u>Cyprus</u> , <u>Czech Republic</u> , Denmark, <u>Estonia</u> , <u>Finland</u> , Greece, <u>Hungary</u> , Iceland, <u>Israel</u> , <u>Latvia</u> , Lithuania, <u>Luxembourg</u> , <u>Malta</u> , Montenegro, <u>Netherlands</u> , <u>Norway</u> , <u>Portugal</u> , Republic of Moldova, <u>Slovakia</u> , <u>Slovenia</u> , Spain, Sweden, Tajikistan, <u>Turkmenistan</u> , United Kingdom and Uzbekistan.	<u>Andorra</u> , <u>Armenia</u> , <u>Azerbaijan</u> , <u>Belarus</u> , Croatia, <u>Cyprus</u> , <u>Czech Republic</u> , <u>Estonia</u> , <u>Finland</u> , Greece, <u>Hungary</u> , Iceland, <u>Ireland</u> , <u>Israel</u> , <u>Latvia</u> , Lithuania, <u>Luxembourg</u> , <u>Malta</u> , Montenegro, <u>Netherlands</u> , <u>Norway</u> , <u>Portugal</u> , Republic of Moldova, <u>Slovakia</u> , <u>Slovenia</u> , Spain, Sweden, Tajikistan, The former Yugoslav Republic of Macedonia, <u>Turkmenistan</u> , United Kingdom and Uzbekistan
Endemic	18 (34%)	Austria, Belgium, Bosnia and Herzegovina, France, Georgia, Germany, Ireland, Italy, Kazakhstan, Kyrgyzstan, Poland, Romania, Russian Federation, Serbia, Switzerland, The former Yugoslav Republic of Macedonia, Turkey and Ukraine.	Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Denmark, France, Georgia, Germany, Italy, Kazakhstan, Kyrgyzstan, Poland, Romania, Russian Federation, Serbia, Switzerland, Turkey and Ukraine.
No report submitted	3 (6%)	Albania, San Marino and Monaco.	

4.2. Regional Reference Laboratory - Berlin update

Prof Annette Mankertz (RRL Berlin)

The Berlin RRL coordinates 19 national laboratories and is particularly invested in molecular surveillance. Currently, the RRL is looking into reinfection cases in vaccinated, asking laboratories with any relevant samples to contact the RRL. In the past year, the RRL has been involved in re-testing, laboratory accreditation and development of the Instand ring trial for molecular detection.

All laboratories had good results for measles and rubella IgM confirmatory testing of up to 30 samples. Similarly, all laboratories scored well in the PT panel for both viruses (as described by Dr Vicky Stambos), although issues were reported with data upload to the website. The RRL is happy to share reference materials (strains, cells, PCR and serology controls) with any laboratory requiring them.

There were high levels of measles circulation in 2015, with a large outbreak of the D8 strain Rostov on Don starting in Berlin in October 2014, which led to a total of 2500 cases and 1 fatality in Germany. The index case belonged to a refugee family from Bosnia and Herzegovina. The virus spread across the refugee population, with young children being the most affected. The outbreak was spread through the general population, due to low immunisation levels. In the wider population,

the most affected were those between 18 and 33 years old. The Rostov on Don D8 strain that caused the Berlin outbreak is now endemic in Germany.

The fatality was of a 4 year-old male, who had been diagnosed with multiple infections. The MMR vaccinations had been postponed due to the fragility of the child, who had been diagnosed with coxsackievirus in November 2014 and parvovirus B19 in December of the same year. The patient developed a respiratory infection with high fever in February 2015, having been taken to the paediatrician and admitted to hospital upon deterioration of health. He then developed an exanthema and was diagnosed with MeV. The infection led to a brain oedema and subsequently to death. An autopsy detected inflammatory cardiomyopathy and MeV in the spleen.

The RRL is collaborating with the RVC in addressing challenges found in linking epidemiology and laboratory data (due to data protection laws) and in obtaining the number of discarded cases. The laboratory was re-accredited in May 2015 to ISO 17025 and ISO 15189 standards with zero deviations and obtained full marks in the Instand mEQA, as well as in serology and NAT. An accreditation visit was successfully conducted by the WHO in March 2016 and the RRL Berlin obtained full marks in the WHO serology PT and in the PCR ring-trial for MeV and RuV detection and genotyping.

The laboratory is assessing the use of HNIG-application (human normal immunoglobulin; passive immunisation). The results obtained so far reproduce the ones published in 2009 by the London GSL and indicate that the products currently used contain sufficient amounts of IgG.

The main challenges faced by Germany for the elimination of measles are the late vaccination of children and the existence of immunisation gaps in adolescents, young adults and healthcare workers.

4.3. Regional Measles and Rubella programme update

Dr Patrick O'Connor (WHO regional office for Europe)

In the past year, there has been an increase in MMR vaccine coverage coupled with a decrease in the incidence of measles. Measles-containing vaccine dose 1 (MCV1) coverage is now just below 95% and MCV2 coverage is over 80%. 88% of measles cases are found in 4 countries and mostly associated with outbreaks: Kyrgyzstan (17779), Bosnia and Herzegovina (4583), Germany (2383) and Kazakhstan (2340). 11 countries reported no measles cases in 2015. Of the 53 member states, 10 reported measles incidences of over 10 cases / million inhabitants.

The majority of cases occurred between January and June, often affecting individuals over 20 years old. The age distribution of cases per country highlights immunisation issues, warranting different immunisation approaches. In Turkey, the majority of cases affected children up to 9 years old, suggesting late vaccination of children. In Italy, Kazakhstan and the Russian Federation, the age distribution of cases indicates low immunisation levels in young children and adults. Other countries' age distribution of cases suggests that vaccination rates are insufficient.

A number of susceptible populations are prone to measles outbreaks: Roma and Sinti communities, susceptible adults, unvaccinated children and adolescents, traveller communities, healthcare workers (HCW), orthodox protestant communities, followers of anthroposophy, and ultra-orthodox

Jewish communities. In many countries, HCWs constitute a significant fraction of outbreak cases (up to 42%). A significant number of outbreaks occur in educational settings.

There were approximately 2500 cases of rubella in EURO, 86% of which in Poland. As for measles, most rubella cases occur in the first half of the year, although this seasonality is less marked for the latter. The incidence tends to be higher in children younger than 10, although an increase in incidence is observed in several countries after 20 years of age.

The main challenges for the EURO are:

- 1) Reach and maintain high vaccination coverage
 - a. Tackle vaccine hesitancy and refusal
 - b. Improve access to vaccination
 - c. Address distrust of vaccination or health authorities
 - d. Strengthen immunisation programmes
 - e. Implement electronic vaccination records and reminders
 - f. Set up recall systems
 - g. Appoint vaccination champions and advocates
- 2) Close immunisation gaps
 - a. Conduct supplementary immunisation activities (SIA)
 - b. Tailor immunisation programmes
 - c. Implement pre-school entry policies
 - d. Put in place policies for HCWs
 - e. Conduct opportunity vaccination
 - f. Provide pre-travel vaccination
- 3) Implement high-quality surveillance systems
 - a. Report suspected cases
 - b. Investigate cases at the laboratory and epidemiology levels
 - c. Carry out laboratory confirmation of cases
 - d. Routinely genotype and sequence samples
 - e. Establish national SOPs for epidemiological and laboratory investigation
- 4) Improve knowledge and training
 - a. Tackle myths and misinformation
 - b. Augment the emphasis on vaccination advantages
 - c. Increase awareness of the disease and complications
 - d. Provide information on vaccines
 - e. Educate the medics, HCWs and scientists
 - f. Provide training in communication to professionals which contact with patients

Although considerable progress has been made towards measles and rubella elimination in the EUR, the region failed to reach the goal for elimination in 2015. In order to achieve and maintain high vaccination cover, close immunisation gaps and uphold good surveillance systems, it is essential to mobilise resources and prompt high political involvement.

4.4. Regional Measles and Rubella LabNet update

Dr Myriam Ben Mamou (WHO regional office for Europe)

There were no new national laboratories added to the LabNet in 2015. However, the setup of a new sub-national network in Italy is underway. The dominating MeV genotypes in EUR were D8 and B3. A large amount of D8 strains had identical N-450 sequence to the Rostov on Don and Cambridge named strains, the latter having been defined in 2016. Fewer samples matched B3 named strains, the majority of which were identical to the Harare strain. The information for rubella is less complete, as few sequences were reported to RubeNS.

57% of states provided data exclusively from WHO-accredited/proficient laboratories in the last verification exercise, which looked at data from 2014. However, 4 countries provided data largely obtained from non-accredited/proficient laboratories. No information was provided on the provenience of the data collected by 13 states and 6 states failed to submit a report on time. Case confirmation is done through IgM detection, which is the golden standard recommended by the WHO for this purpose. However, some states report using only molecular detection for case confirmation, an approach that is limited in that it does not guaranty sensitivity.

Most countries (47) submitted their ASU report in time for the RVC meeting of October 2015. 37 states reported measles cases, 33 of which provided genotype information. Overall in EUR, 69% of the chains of transmission and 80% of sporadic cases were genotyped. 22 member states (MS) reported rubella and 2 CRS cases. Only 2 MS provided RuV genotype information.

In 2015-16, the EURO facilitated the roll-out of a mEQA exercise for the region developed by Instand and the RRL Berlin, conducted the annual accreditation review for the LabNet, supported capacity-building activities through laboratory visits, developed the new MRLDMS2 application, organised meetings, training and contributed to the annual verification exercise, conducted an EUR MR LabNet consultation on serosurveys for ETAGE, and provided reagents for a number of MS. Accreditation visits are now increasingly conducted in conjunction with epidemiologists from WHO and with RVC representatives so that they are more comprehensive.

Over 25 000 samples were tested in 2015 for measles and rubella, with a higher number of positive samples for measles. Only 19% of countries reported IgM results within 4 days of sample reception as recommended by the WHO. The majority of reference laboratories tested the required amount of samples yearly and performed proficiently in the serology PT. However, only 63% of participating laboratories passed rubella molecular EQA, and, only 30% report genotyping results to MeaNS and RubeNS in a timely manner. 88% of reference laboratories report to CISID or MRLDMS, 53% doing so timely and 74% providing complete data. Only approximately half of the laboratories have robust IQC procedures in place.

Training will be put in place to support laboratories in the genotyping of RuV. All LabNet members are asked to report sequence information to MeaNS and RubeNS in a timely manner. 9 MS reported measles cases, but did not submit sequences to MeaNS. Only 9 laboratories submitted measles and rubella sequences on time to the appropriate database. The laboratories should seek advice when suffering technical difficulties and start collecting appropriate specimens when they do not do so already.

The main challenges faced by the EUR LabNet are connected with timeliness and completeness of reporting, implementation of internal quality control procedures, timely reporting of IgM results to the surveillance system. The verification process is going well for measles, but needs improvement

for rubella, especially in terms of RubeNS sequence submissions. Laboratories must be made aware of the sensitivity issues associated with using a non-IgM-based strategy for case confirmation. The LabNet will need to ensure continuing funding and political commitment to guarantee the high-quality results that are key to the elimination of measles and rubella in the EUR.

4.5. WHO global program and LabNet update

Dr Mick Mulders (WHO Headquarters)

The number of measles cases worldwide markedly decreased until 2008, but appears to have stabilised since. This could be due to an overestimation of cases associated with the improvement of surveillance systems. MCV1 coverage has reached 83% and coverage of MCV2 is steadily increasing, being currently over 50%. 20% of countries have MCV1 coverage lower than 80%, with the AFR being the most challenging. Since 2008, 32 countries have introduced a routine MCV2 immunisation.

Large outbreaks occurred between May of 2015 and April of 2016, namely in Mongolia and Nigeria. While in the latter mostly children were affected, in Mongolia the incidence rate was higher in 18-30 year olds. SIAs were implemented to address immunisation gaps and stop transmission. 63% of the 581 290 measles cases reported between 2011 and 2016 occur in 1-15 year olds, mainly in unvaccinated individuals.

The coverage of rubella-containing vaccine (RCV) has been steadily increasing and reached 46% in 2014. Again, vaccination coverage is lowest in the AFR. The majority of congenital rubella syndrome (CRS) cases are found in the AFR and SEAR. 17 countries have introduced routine RCV between 2012 and 2015 and a further 16 are planning to introduce rubella vaccination between 2016 and 2018.

A high level of measles control is being maintained, with over a million measles-related deaths being averted each year. However, there are large outbreaks on-going and progress towards elimination has slowed, in part due to over 6 years of stagnation in vaccination programmes. Although rubella elimination has been verified in the AMR and there has been an increase in RCV coverage, over half of the world's children remain unvaccinated. A midterm review will be conducted to understand why, with the exception of the AMR, all other regions are off-target for achieving measles and rubella elimination.

The MR elimination programme should take advantage of new opportunities arising from Gavi, GHS and the transition of resources from the polio eradication programme. The quality and coverage of current surveillance and vaccination programmes must be improved on. In order to accelerate regional elimination efforts, countries must have a sense of ownership, promote political commitment and engage adequate resources.

The GMRLN is the largest globally coordinated laboratory network, composed of 703 laboratories: 3 GSLs, 14 RRLs, 180 NRLs and 506 sub-national/provincial/prefecture laboratories. It provides high-quality laboratory support for measles and rubella surveillance. The data collected by the network is essential to measure progress towards elimination. The continued expansion of the GMRLN is necessary to maintain surveillance, not only of MR, but also of other vaccine-preventable diseases and emerging pathogens.

The main tasks of the GMRLN include case confirmation, gathering sequence information, providing evidence for verification of elimination, building capacity and providing training, establishing and

monitoring laboratory performance, and supporting studies of population immunity. In order to perform well, laboratories must report complete data in a timely fashion, participate in proficiency testing exercises, have a robust internal and external quality control procedure in place and meet the WHO performance indicators.

The number of samples tested for both measles and rubella IgM has suffered an overall increase since 2006. In contrast, the number of laboratories participating in the annual EQA for serology testing has remained relatively constant for all regions, except for the WPR, which has seen an increase in participating laboratories. The SEAR and WPR were the regions performing best in 2015.

The number of sequences submitted to MeaNS and RubeNS is increasing, although much higher for the former. The most common circulating measles strains in the 2010-5 period belonged to genotypes B3, D4, D8 and H1. Genotyping information is a valuable asset in informing the programme on how well it is doing. Some genotypes, such as B2, D11, D5, D6 and D7, are no longer reported, suggesting that the diversity of circulating MeV is decreasing due to the interruption of chains of transmission. The overall picture is less positive for RuV, with insufficient sequences being obtained and vaccine-related rash being reported instead of real cases.

New developments are being assessed/implemented such as an alternative sequencing window for MeV to improve outbreak characterisation, an oral fluid collection device to be used on the field, a point of care test for measles IgM and Luminex technology for multiplex serology assays. The main challenges faced by GMRLN are to manage an increasing workload derived from testing demands and competing priorities, and the retention of expertise in face of the growing requests for more complete laboratory testing.

The WHO has declared poliovirus type 2 eradicated and is discontinuing the use of this type in polio vaccines. Hence, any future polio type 2 cases can only result from laboratory infections or vaccine virus. For this reason, GAPIII requests that all samples containing or potentially containing poliovirus type 2 or Sabin 2 are destroyed. This includes any respiratory or faecal samples, as well as cell culture-derived isolates. Thus, all except serum samples collected by the LabNet for MR testing are affected.

The laboratories are given 3 options:

- 1) Destroy all relevant samples (major implication for sample archives);
- 2) Contain samples in polio-essential facilities (PEF) (enhanced BSL3 containment);
- 3) Transfer samples to a PEF.

A fourth option is being considered in which infectious materials may be inactivated so that they are no longer infectious and no longer contain full-length RNA or cDNA. All laboratories will be required to fill in an inventory form for polio.

4.6. MeaNS and RubeNS update

Dr Kevin Brown (GSL UK)

Dr Kevin Brown updated the participants on the MeaNS and RubeNS databases. In the last year, Dr Richard Myers has changed post and is no longer in charge of the databases' development and curation. Two new posts will be appointed and candidates have been selected. Dr David Williams is expected to take up Richard's position in August. MeaNS can be found at www.who-measles.org and RubeNS' website is www.who-rubella.org. Currently, there are 29884 viral sequences in MeaNS and 1540 in RubeNS.

A significant number of MeV genotypes have not been seen in 5 years: B2, D5, D6, D7 and D10. The data in MeaNS is not fully representative, given that it is biased towards the genotypes most frequently found in the countries that submit the majority of sequences. In 2015, the WPR was the region that submitted most sequences to MeaNS (3185), followed by the EUR (960) and the AMR (240). The majority of sequences submitted belong to measles genotype H1, which is circulating in the WPR and has been exported to several other regions. Sequences of genotypes D8 and B3 are the second and third most common in MeaNS, being the two most common for EUR submissions, while there were very few submissions of D4 sequences.

A very significant delay in sequence submission is observed, which limits the usefulness of the data. Laboratories should submit their data within 8 weeks of sample reception as recommended by the WHO, although the earlier the submission is done the more helpful it is. A large amount of measles B3 Harare has been circulating in the EUR, but the number of sequences is limited compared to 2012 and 2014, having been overtaken by genotype D8 in terms of number of sequences submitted.

Although the number of RuV sequences uploaded to RubeNS has been increasing, it is still too low, limiting the usefulness of the data collected. The EUR LabNet has submitted only 9 different RuV strains in 2015, which is most likely not representative of the rubella circulating in the region.

MeaNS and RubeNS are essential tools for evaluating virus circulation and diversity. Issues with submission bias and timeliness limit the usefulness of the data, particularly for RubeNS. The upload of data to the databases is required for verification of each country's elimination status, hence timely and representative submission of sequences to MeaNS and RubeNS acquire additional importance.

4.7. Public Health England – Global Specialized Laboratory update

Dr Kevin Brown (GSL UK)

Since 2013, Wales, Scotland and Northern Ireland have separate health authorities, but report to the WHO through Public Health England (PHE). PHE is now part of the Department of Health and subject to public funding, being responsible for public health, health inequalities and vaccine procurement/delivery. Major restructuring and cost saving actions are on-going and a new Clinical Services Unit has been created. A National Infection Service has been formed, instituting more close collaboration between laboratories and epidemiology. The future of the Virus Reference Department (VRD) is uncertain. Urgent measles PCR testing is now carried out in regional laboratories. In Scotland and Wales, PCR is the only testing in use. Further uncertainty derives from the recent Brexit vote, as the repercussions for public funding and the structure of health services are unknown.

Changes in the department mean that Dr Richard Myers (senior bioinformatician) and Mrs Heather Lawson (technical manager) have moved on to different positions. Measles and rubella IgM/IgG/total IgG and RT-PCR testing will now be carried out by CSU, while the specialist testing, such as genotyping, avidity and cell culture, will remain the responsibility of the current unit. Sadly, Dr Dhan Samuel died in March 2016 after a short illness. He was an outstanding serologist in VRD, having led the development of oral fluid collection devices and the recent point of care test for measles IgM.

MCV coverage is increasing and reaching good levels, reaching almost 95% for MCV1 and 89% for MCV2. The number of measles cases was very high throughout 2013 due to a large outbreak in England and Wales, but following a very successful catch-up campaign carried out in response to the outbreak, has been drastically reduced from 2014 until present. The measles strain associated with the outbreak, D8 Taunton, was last detected in March of 2014.

Since then, the majority of cases were from genotype B3, until February of 2016, when an imported D8 case triggered a new outbreak centred in London. Most of the cases occurred in young adults born outside the UK and who had unknown vaccination status. A campaign targeting adults for vaccination was initiated. Although no B3 or D8 strains have circulated for more than 12 months, the D8 Cambridge strain associated with the London outbreak has been exported worldwide, namely to Belgium, Spain, Italy and the USA.

In the aftermath of the excessive workload resulting from the 2013-14 measles outbreak in England and Wales, PHE initiated the rollout of measles PCR testing to Public Health Laboratories (PHL). After 6 months, some issues have been found, one of which is the reduced number of oral fluid samples received by PHE for surveillance purposes. The rollout will be re-evaluated after the WHO accreditation and cellular controls will be added to the assay. It must be highlighted that negative cases cannot be discarded in the absence of a cellular control.

A PCR proficiency panel was produced in 2015. It consisted of 10 samples, 9 of which were measles positive and 1 was negative. The panel was dispatched to all PHE laboratories as well as to laboratories in Scotland, Wales and Northern Ireland. It was found that only two PHE laboratories use cellular controls. Northern Ireland did not employ cellular controls, but sends all samples to PHE. The use of PCR controls for measles detection is under review. There were issues obtaining data from Scotland, although no measles cases are known to have occurred there.

A significant amount of rubella testing has been conducted, but the majority of samples were negative. In order to achieve the quota for rubella surveillance tests, all measles samples are now tested for rubella. However, laboratories are having difficulty in understanding why they should be carrying out RuV testing given the extremely low numbers of samples received from rash illness surveillance. Currently, all observations indicate that there is no endemic rubella circulating in the UK. There were three cases of CRS in 2015, all in non-UK born individuals.

Issues have been found with the RuV IgG screening during pregnancy. The absolute value obtained from the assay is significantly lower after vaccination than after wild-type infection and hence, given the reduction in rubella circulation, an increased number of patient samples yield results closer to the assay cut-off value. For these reasons, it was decided to stop the screening program in pregnancy and verify the vaccine history after delivery instead.

Following the success of the catch-up vaccination campaigns carried out in response to the 2013-14 measles outbreak in England and Wales, there have been fewer cases of measles in the UK. In this time, it has been found that the number of oral fluid (OF) samples yielding reactive or equivocal results in the Microimmune measles IgM assay has been increasing. It has been observed that reactive samples are often associated with recent vaccination or constitute false positives (based on PCR or IgG assay results). In order to investigate this, 90 samples from 2012 (prior to the detection of the current issues) were re-tested. The results indicate that the Microimmune measles IgM assay is not performing as previously for OFs, which could be caused by a variety of alterations introduced since the test was adopted, including in sample collection, extraction and controls. The selected OFs were sent to Microimmune for further investigation.

The London GSL laboratory has been evaluating the utility of employing an extended sequencing window for the characterisation of chains of transmission. It was found that using the non-coding region between the matrix and fusion genes (MF-NCR) or sequencing the whole of the measles genome, except termini (WGS-t) provides further information on phylogeny that may be helpful for well-resourced countries approaching elimination. The N-450 region is still the standard for genotyping and outbreak investigation, but these alternative regions could be used to complement the information routinely gathered using the N-450.

155 measles isolates have been deposited in the strain bank, 11 of which in 2013. In 2015 the UK was the only country submitting isolates to the strain bank. There are 17 isolates in the rubella strain bank, including a wild-type virus from London 2007 and 14 isolates submitted by the CDC in 2008. Many of the samples are from CRS cases, rather than from circulating virus. The polio GAPIII initiative may have major implications to strain archives and potentially strain banks. The laboratories are encouraged to submit their strains to PEFs.

There have been issues with obtaining samples back for re-testing from some laboratories. Increasing wishes for devolution of Scotland and Wales mean that some samples are only tested by PCR, which has limitations. The communication between regional laboratories and national epidemiologists needs improvement in order that case clusters such as the one occurred in London are addressed more efficiently. A major information campaign is on-going concerning the cessation of rubella screening in pregnancy. Austerity cuts as well as changes brought in by restructuring, prioritisation and Brexit may threaten the measles and rubella work carried out in the London GSL.

4.8. Regional Reference Laboratory - Luxemburg update

Dr Judith Hübschen (RRL Luxemburg)

There are 22 countries in the RRL Luxemburg constituency, 4 of which have no national laboratory. In 2015, 25 serum and 12 molecular proficiency panels were distributed in the constituency, as well as 319 filter paper samples and 30 Microelute cards. CDC kits were also sent to NRLs. The RRL is increasingly using filter paper for shipment of samples for proficiency testing, confirmatory testing and genotyping as this expedites and simplifies transit of samples across a large region.

The laboratories used four different kits for MeV and 5 for RuV IgM testing. All laboratories performed well, with 17 out of 19 obtaining a pass score, 4 of which achieving full marks for measles and 7 for rubella PT. The major concerns are that not all laboratories used the correction factors

indicated for the kit employed and few laboratories had in-house controls in place (6 for MeV and 8 for RuV).

21 laboratories participate in confirmatory testing, the prevalent sample types now being dried blood spots and FTA serum. All laboratories had 100% concordance in confirmatory testing, although five laboratories sent fewer than 10 specimens for re-testing. Full concordance was observed for RuV confirmatory testing too, but 3 laboratories did not send any samples and another 3 sent fewer than 10 specimens.

Measles specimens were received for genotyping/sequencing from DRC, Serbia, Bosnia and Herzegovina and Georgia. The prevalent measles sequences were from the D8 genotype, Rostov on Don and Frankfurt Main strains. Extended sequencing carried out on 22 urine samples of confirmed measles patients received from the NRL Israel detected a maximum of a single nucleotide difference in the P gene and 5 in the H gene, with 8 sequence variants found; sequencing of the MF-NCR is ongoing. One of four clinical samples from two rubella patients sent in by the Portuguese NRL was positive for RuV. This was a CRS case of genotype 2B.

The RRL Luxembourg was involved in accreditation visits to the two NRLs in Bosnia and Herzegovina and the NRL Georgia, and collaborated with the Serbian and Bosnia and Herzegovina NRLs in the production of outbreak reports, in seroprevalence studies with Laos and in capacity-building for the NRL Serbia.

4.9. Regional Reference Laboratory - Moscow update

Dr Sergey Shulga (RRL Moscow)

The RRL Moscow's constituency includes 24 laboratories: the RRL, 10 NRLs and 13 are sub-national laboratories. All laboratories participated in the measles and rubella IgM PT panels and obtained 100% concordance of results, except for 2 laboratories, which did not receive the panels and 1 that has not submitted results. Issues with data submission have been reported.

Some laboratories are sending too few or no positive samples for measles IgM confirmatory testing, an issue that is heightened for rubella. One laboratory sent no samples for confirmatory testing. All the samples that were sent for MR confirmatory IgM testing obtained concordant results. The laboratories were reminded that all IgM-positive samples should be sent for confirmatory testing at least twice a year.

In 2015, the RRL Moscow provided laboratories with equipment, reagents and supplies, and ELISA serum controls and panels. It also organised training activities in collaboration with the WHO and the CDC, and organised/participated in joint meetings with epidemiologists, clinicians and laboratories, and with the NIS surveillance services chiefs. A new database is being developed to collect ELISA, PCR and genotyping data for measles, rubella and CRS suspected cases.

Most of the measles cases were associated with several small outbreaks linked to importation, with 78 cases imported from 17 countries and then spreading amongst susceptible populations such as unvaccinated adults and young infants, Roma community and religious groups. The majority of strains detected in the constituency were of the D8 genotype. Only Ukraine and Tajikistan have no genotyping data. RuV sequence data indicates that this virus is not endemic in the Russian

Federation, as all the detected strains are closely related to various imported strains. There has been a single CRS case reported in 2015. No surveillance system during pregnancy is in place.

The challenges for the RRL Moscow are to promote rubella genotyping in the region, prepare for ISO 15189 accreditation, organise samples for panel shipment, and address a high staff turnover.

4.10. Selected poster presentation – NRL Croatia

Dr Tatjana Vilibic-Cavlek and Dr Jelena Ivancic Jelecki (NRL Croatia)

The poster produced by the Croatian laboratory was selected as the best amongst those exhibited during the meeting. The NRL Croatia representatives, Drs Tatjana Vilibic-Cavlek and Jelena Ivancic Jelecki, were invited to present their work to the participants.

Measles vaccination was introduced in Croatia in 1968, consisting of the MMR since 1976. There is currently over 94% coverage for the MCV1. Before measles vaccination was introduced, 5 000 - 20 000 cases were reported in Croatia per year. In the last two decades, the number of annual cases of measles notified had been drastically reduced and is below 10, with the exception of outbreaks resulting from importations in 2003-4, 2008 and 2014-15. Four SSPE cases were reported in 2002.

For rubella, except for an outbreak in 2007, only sporadic cases have been reported. A recent seroepidemiological study (2005-9) has shown that 94.6% of women in childbearing age are immune to RuV. However, a fall in immunisation levels raises concerns over rubella immunity.

The measles outbreak of 2014-15 started in December of 2014 and led to 220 cases distributed in 17 clusters by March of 2015. The majority of cases were in the Roma population due to very low vaccination coverage (less than 50%). Sporadic cases were identified in the general population, with 6 cases detected in HCWs. Sequencing indicated that the outbreak was caused by a single measles D8 strain. The outbreak affected all age groups, mainly those with no or incomplete vaccination record.

During 2015-16, 164 samples were PCR tested for measles, 105 of which positive. 30 of 50 samples tested for measles IgM were positive and none of the 50 samples tested for rubella IgM were positive. One MeV isolate was obtained in this period of time.

The NRL Croatia collaborates with the University of Zagreb for measles sequencing. N-450 sequencing for two decades shows that the circulating strains detected in Croatia are identical to contemporaneous epidemiological data from other European countries, with measles D4 epidemics in 2004 and 2008, a single B3 import-related case in 2014, D8 outbreaks in 2014-15 and an imported D8 case from Germany in 2016.

As a result of high vaccination coverage for the past few decades, Croatia has experienced low numbers of measles and rubella cases. However, the decrease of immunisation rates in the last four years may lead to the reintroduction of measles and/or rubella in the future.

3. Summary and recommendations

The following recommendations result from the exchanges and discussions between the participants throughout the meeting:

Serology

1. All laboratories are encouraged to use the same kit consistently for routine testing and proficiency testing.
2. Unmet 4-day turnover time for MR serology testing to be mitigated with timeliness of positive RT-PCR result within 4 days, as appropriate.
3. The new laboratory manual should include validated ELISA protocols and comparison of available kits, to support laboratories in the selection of high quality reagents.
4. There have been issues with false positives detected with the Microimmune measles IgM oral fluid kit. Laboratories using this kit should contact the global specialised laboratory in London.
5. Laboratories should be provided with a clear list of samples that should be sent for retesting.

Molecular surveillance

6. Discard of cases based solely on negative molecular testing results is strongly discouraged.
7. All laboratories, including those that do not carry out the sequencing themselves, should submit sequences that match the requirements to MeaNS or RubeNS in a timely manner (within 8 weeks).
8. Laboratories are encouraged to use the tool available in MeaNS and RubeNS to produce the line listings for their annual status update (NVC report to the RVC). It is recommended to use the functionality "NL listing" available in MeaNS.
9. If laboratories wish to so submit their sequences to GenBank, they are encouraged to use the tool available for the effect in MeaNS and RubeNS.
10. A spreadsheet for bulk sequence submission to MeaNS and RubeNS is available on request from means@phe.gov.uk
11. MeaNS and RubeNS training slides as well as country ISO codes for WHO strain naming will be made available to those interested.
12. Laboratories should send sequencing to their RRL if they do not sequence regularly or are not accredited for sequencing.
13. Standard sequencing methods will be included in the next version of the laboratory manual (expected for January 2017).
14. All laboratories interested in or carrying out extended sequencing or NGS are welcome to participate in the NGS and extended sequencing workgroup (NEW). Please e-mail Mick Mulders or Alberto Severini.

Internal quality control

15. It is recommended that all laboratories have robust IQC protocols in place.
16. Laboratories will be supported in the implementation of Westgard monitoring of tests. If required, training can be provided by the WHO or RRLs.
17. The possibility of establishing a range of permissible standard deviations to use in Westgard will be investigated.
18. If any laboratory receives insufficient samples suitable for use as IQCs, they should contact their RRL or RLC for support.
19. RRLs to advise the laboratories in their constituency on commercial reagents that can be used for IQC, in particular for rubella.
20. The annual accreditation review will include feedback on the laboratories' IQC processes.
21. Laboratories wishing to obtain ISO15189 accreditation are advised to seek advice from WHO and those laboratories that have already achieved accreditation.

Proficiency testing

22. Instructions on the login and online result submission process for PT will be available on the website.
23. The GSL/RRLs are asked to provide VIDRL with a list of laboratories that should be sent a proficiency test panel.
24. Laboratories unable to login to VIDRL's website for PT results submission should email VIDRL at measlepanel@mh.org.au and request assistance to ensure login is possible in preparedness for the next panel.
25. Laboratories are encouraged to provide direct feedback on the web-based reporting of PT results at measlepanel@mh.org.au.
26. VIDRL to copy RRLs when sending PT results reports.
27. Laboratories able to provide serum samples that are IgM positive for measles, rubella or parvovirus B19 for use in future PT panels, to communicate with RLC for coordination of shipment to VIDRL.
28. An option will be introduced for saving submission as a PDF file in future versions of the PT website.
29. The wording in the PT website for non-Siemens kits will be reviewed to ensure clarity.
30. In the future the PT website will be updated to allow for introduction of correction values for non-Siemens tests. In the meantime, laboratories should input all the relevant information in the comments box provided.

Molecular EQA

31. Laboratories accredited for sequencing or carrying out sequencing on a regular basis should participate in the molecular external quality assessment scheme provided by Instand e.V.. The GSL, RRLs and NRLs should participate the WHO option of the scheme and the remaining laboratories in the regular version
32. Laboratories not carrying out regular sequencing in routine or not accredited for sequencing should consider closed mEQA schemes or the CDC practice panel for training purposes (contact RLC).
33. RRLs to specify laboratories who want to participate in detection and/or genotyping of measles and rubella for the next mEQA scheme. The RRLs should ensure that their constituency laboratories are prepared for the next mEQA.
34. Laboratories will be asked to identify the software they use for chromatogram analysis in the next mEQA scheme.
35. A letter with detailed instructions for the next mEQA will be sent by WHO EURO to all laboratories as soon as possible.
36. Laboratories should contact their RRL if they have issues with their mEQA exercise.
37. mEQA provider will inform participating laboratories, RRLs and RLC of mEQA results.
38. WHO remains committed to support laboratories that are facing difficulties in financing their mEQA exercise.

Reporting to WHO

39. All transmission chains, independent of length, must be reported to the WHO.
40. All NLs, including RRLs and GSL, should appraise themselves of the new MRLDMS2 database and plan to implement reporting of positive cases when the live site is launched.
41. Reporting to MRLDMS2 will be at least monthly in the first instance, with zero reporting if no samples received during the reporting month.
42. Laboratories that use their own LIMS will be able to implement bulk upload to MRLDMS2.
43. Those laboratories that have a LIMS system in place can either:
 - a. Use bulk upload to input all samples into the MRLDMS2 database, in which case they may discontinue the monthly upload of aggregate data to CISID;

- b. Use bulk upload to input positive, suspected and confirmed cases into MRLDMS2 and submit aggregate data on negative samples to CISID on a monthly basis. These laboratories must be able to demonstrate that the data collected for negative samples is representative (e.g., includes all age groups).

Laboratories can modify their choice in the future.

44. Laboratories that do not have a LIMS system implemented will be able to use the MRLDMS2 database as one.
45. When laboratories use the MRLDMS2 database as a LIMS system, all samples (including negatives) should be input into the database and reported.
46. Reporting through MRLDMS2 and CISID will be kept under review.
47. Redundancies between the MRLDMS2 and TESSy databases should be identified and resolved.
48. MRLDMS2 users are asked to provide feedback on the website, prior to its launch, to the RLC.

Verification

49. Laboratories should apprise themselves of NVC requirements and prepare their report accordingly.
50. MeaNS distinct sequence ID and RubeNS sequence ID are required in the verification report.
51. Laboratories and epidemiologists should communicate in order to harmonize data and definitions prior to submission of the country's report to the NVC.
52. All 2015 sequences should be reported to MeaNS and RubeNS in time to the next NVC report (due 31st of July 2016).

Training

53. The video produced by CDC on sequence analysis will be available on the MeaNS website.
54. A need for technical support in the detection, sequence analysis (interpretation of chromatograms) and genotyping of rubella was identified in the 2015 mEQA. Training (potentially e-learning) will be provided to address this.

Surveillance

55. Surveillance and outbreak capacity must be ensured. Laboratories should communicate with their RRL or WHO to address any issues.
56. During outbreaks, testing should be done only on representative samples to preserve laboratories' resources.
57. WHO to consider the possibility/advantage of establishing global case and outbreak definitions.
58. Collaboration between laboratories and epidemiologists should be strengthened to improve reporting and explore the advantages of wider surveillance systems (e.g., rash and fever).
59. Relationships with epidemiologists and public health policy makers should be improved so that laboratory findings on, for example, vulnerable groups are followed up.
60. GSLs, RRLs and the WHO should discuss the use of molecular epidemiology to support decision-making regarding elimination status.

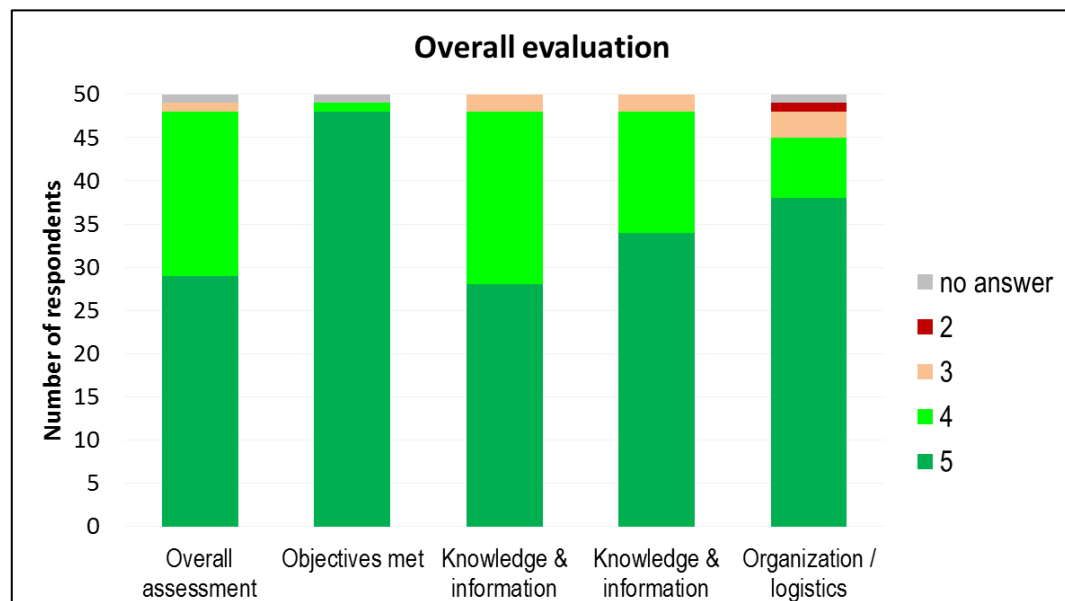
Polio containment

61. The WHO should provide laboratories with a detailed list of all samples affected by the polio containment exercise.
62. All laboratories will need to fill in an inventory of polio-containing/potentially containing materials.
63. The options being proposed/considered for laboratories who hold samples affected by polio containment are:

- a. Destruction of (potentially) contaminated materials;
 - b. Holding samples in polio-essential facilities (PEF) – enhanced BSL3 laboratories; if the laboratory is not a PEF, specimens can be transferred to a PEF;
 - c. Inactivation of materials so they are no longer infectious and no longer contain full-length RNA or cDNA (being considered – no method approved or discussed yet).
64. Non-polio laboratories should be given needs-oriented training by the WHO.

Annex : Evaluation of the meetings by the participants (both groups, 27 June – 1 July 2016)

Rating is on a 5-point scale, 1 is the lowest and 5 the highest possible.



Some quotes from participants

"Very useful and practical work and very useful poster session".

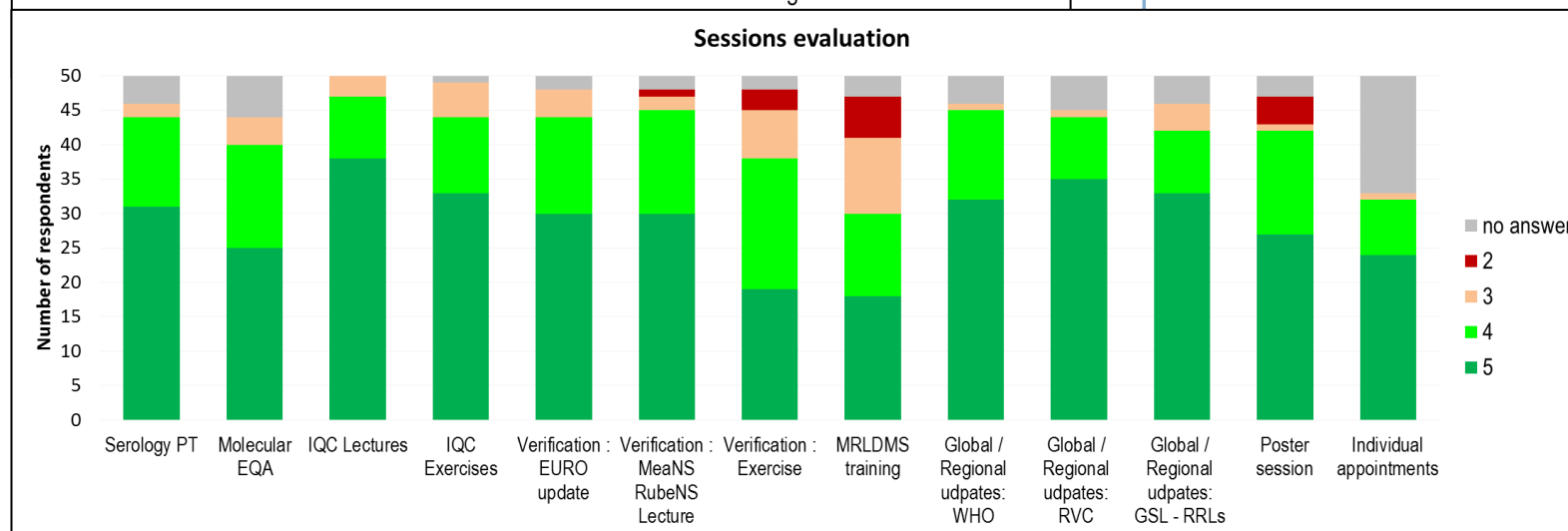
"Poster presentations were better than individual presentations.
Format was good and venue amazing."

"Ensure better internet connection, especially for online exercises."

"Large volume of information!"

"Practical format of training sessions and exercises should continue to be used."

"E-posters should be continued but readability and quality of posters should be increased, perhaps little more time for poster session would be better."



"Practical sessions are useful!"

"For next meeting, plan for more discussion of points on national laboratory reports with a focus on issues in practice, more group training, and discussion of testing and reporting of CRS."

"I was happy with everything!"

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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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