3-4 FEBRUARY 2015 // LONDON, UNITED KINGDOM

10th Meeting of the Measles/ Rubella Regional Reference Laboratories of the WHO European Region

World Health Organization
REGIONAL OFFICE FOR Europe

MEETING REPORT

10TH MEETING OF THE MEASLES/RUBELLA REGIONAL REFERENCE LABORATORIES OF THE WHO EUROPEAN REGION

LONDON, UNITED KINGDOM, 3–4 FEBRUARY 2015

KEYWORDS

ACCREDITATION
COMMUNICABLE DISEASE CONTROL
DISEASE ELIMINATION
SURVEILLANCE
EPIDEMIOLOGY
IMMUNITY
LABORATORIES
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
DRC	Democratic Republic of the Congo
ECDC	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
EUVAC	European surveillance network for selected vaccine-preventable diseases
GMRLN	global measles/rubella laboratories network
GSL	global specialized laboratory
HH6	human herpesvirus type 6
IQC	internal quality control
IgG	immunoglobulin G
IgM	immunoglobulin M
Labnet	laboratory network
MCV	measles-containing vaccine
MeaNS	Measles Nucleotide Surveillance Database
MeV	measles virus
MMWR	Morbidity and Mortality Weekly Report (CDC)
MR	measles/rubella
MRLDMS	Measles and Rubella Laboratory Data Management System
NGS	gext generation sequencing
NIS	newly independent states
NL	national laboratory
NRL	national reference laboratory
NSP	non-structural protein
NVC	national verification committee
OF	oral fluid
PAHO	Pan-American Health Organization
POCT	point of care test
PCR	polymerase chain reaction
PHE	Public Health England
PHL	public health laboratories
POCT	point of care test
PRN	plaque reduction neutralization
PP	proficiency panel

PT	proficiency test
RAGIDA	risk assessment guidelines for infectious diseases transmitted on aircraft
RLC	Regional Laboratory Coordinator
RKI	Robert Koch Institute
RNA	ribonucleic acid
RRL	regional reference laboratory
RubeNS	Rubella Nucleotide Surveillance database
RVC	Regional Verification Commission for Measles and Rubella Elimination
SAGE	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
VIDRL	Victorian Infectious Diseases Reference Laboratory (Melbourne, Australia)
WER	Weekly Epidemiological Report (WHO)
WGS	whole genome sequencing

Acknowledgements

The WHO Regional Office for Europe gratefully acknowledges Public Health England for its support and collaboration in hosting this meeting.

Executive summary

The 10th meeting of the measles/rubella regional reference laboratories (RRLs) of the WHO European Region was held on 3–4 February 2015 in London, United Kingdom.

The meeting was attended by representatives of the following institutions/laboratories:

- national reference laboratories (NRLs)
    - CNR Rubeole, Hopital Universitaire Paul Broussse; Villejuif; France
- European measles/rubella regional reference laboratories (RRLs)
    - Robert Koch Institute, Berlin, Germany
    - Luxembourg Institute of Health, Luxembourg City, Luxembourg
    - Gabrichevsky G.N. Research Institute of Epidemiology and Microbiology, Moscow; Russian Federation
- RRL for Pan America Region
    - Public Health Agency of Canada, Winnipeg, Canada
-
- global specialized loaboratories (GSLs)
    - Public Health England, London, United Kingdom,
    - Centers for Disease Control and Prevention (CDC), Atlanta, United States of America
- European Centre for Disease Prevention and Control (ECDC)
- WHO
    - headquarters
    - Regional Office for Europe.

Updates were given on progress towards implementation of previous meetings' recommendations, and ongoing issues and projects were discussed.

The following key recommendations were agreed upon by the participants.

1. Accreditation

1. Proficiency testing (PT) panel results: WHO Labnet should apply a more stringent scoring system to PT results. The Victorian Infectious Diseases Reference Laboratory (VIDRL) is requested to test several proposals against current 01404 PT, to share for discussion and agreement on the final scoring system to be applied in the next round.
2. Retesting: To enable full implementation of the 2014 recommendation, the RLC should circulate the revised retesting form as well as the updated instructions for harmonization between the WHO Regional Office for Europe RRLs. RRLs agreed on getting samples for retesting from suspect cases only, even if the number is less than 20 (see corresponding recommendation from 2014 RRL meeting).
3. Molecular PT: it is recommended to roll it out for all NRLs providing independent results for molecular testing (WHO, CDC, RRL Berlin)

4. The Measles and Rubella Laboratory Data Management System (MRLDMS) has been upgraded with additional functionalities: RLC to expedite finalization and RRLs to collaborate for piloting.

2. ELISA comparative studies

5. It is recommended to set up a working group to further discuss and agree on the principle of conducting ELISA comparative studies, and define future steps regarding the design and implementation of these studies (GSLs, RRLs, WHO)
6. If the decision to conduct enzyme-linked immunosorbent assay (ELISA) comparative studies is confirmed, the panel and protocol should be well defined and questions to be answered should be agreed (ELISA studies working group).
7. It is recommended to take the opportunity of the upcoming revision of the measles and rubella laboratory manual to integrate a section providing comprehensive guidance on kit selection (Manual revision working group).
8. Informative data from kit comparison have been made available from PT panels. It is recommended that this data be summarized and disseminated (GSL London, WHO headquarters).

3. Molecular detection/surveillance

9. PCR as an exclusive tool for measles cases classification should be a possible option for NRLs (serology stays as the first option). It is requested to provide comprehensive guidance in the lab manual for quality PCR: which samples, time of collection, IQC procedures, molecular PT (Manual revision working group).
10. In order to generate more genotyping data, countries are encouraged to use FTA® when appropriate.
11. Whole genome sequencing results are promising, GSLs and RRLs are encouraged to continue investing and exploring this tool in their research and development activities. However, this technology is not recommended for wide use in the European Region now.

4. Verification of elimination

12. Reference laboratories are urged to increase timeliness and completeness of reporting to WHO nucleotide surveillance databases MeaNS and RubeNS: all measles and rubella sequences generated should be submitted in a timely manner.
13. Provide appropriate guidance to NVCs for the development of country annual status updates: increase integration of epidemiological data with sequence information to inform the Regional Verification Commission (RVC), WHO Secretariat on virus transmission pathways.
14. Seroprevalence studies: circulate the draft of WHO global guidelines on seroprevalence studies among RRL meeting participants for comments, labnet to provide expert guidance on how to conduct quality seroprevalence studies (seroprevalence/immunity, assays, samples).

5. Capacity building/training

15. Opportunities should be provided for laboratory training and capacity building. Training approaches and content should be tailored to training needs, to be identified from the performance perspective (WHO, RRLs, GSLs).
16. It is recommended to use existing mechanisms and sources of information to assess training needs: accreditation check-lists and on-site visits, verification process and documents, GSL /RRL expertise, environment analysis (WHO, RRLs, GSLs).
17. When developing trainings, organizers are recommended to ensure coordination and harmonization and make use of existing resources, including e-learning options available from CDC labs (WHO, RRLs, GSLs).

6. Publications

18. The global Measles/Rubella Laboratory Network (MR Labnet) is strongly encouraged to publish in upcoming issues of Morbidity and Mortality Weekly Report (MMWR)/ Weekly Epidemiological Record (WER), a comprehensive update on measles genotyping, including guidance about the strategy of genotyping during outbreaks, and approaches to identify separate clusters (WHO, GSLs).
19. The European MR Labnet is strongly encouraged to publish papers on the specific features of the Region in the context of elimination: progress and issues of the Region (chains of transmissions, genotype replacement). Additionally, regional publications with innovative ideas or new perspectives on existing data are welcome (RRLs, GSLs).
20. Seroprevalence studies from Russian Federation have been published earlier in Russian. Publication of these data in English journals will be highly appreciated, as this would allow wider dissemination and use (RRL Moscow).
21. There is a need to publish a paper on MeaNS. A proposal should be presented (to MeaNS/Rubens Steering Committee) at the global MR labnet meeting in June 2015 (GSL, WHO).

1. Introduction

The Measles/Rubella Laboratory Network (MR Labnet) of the WHO European Region was established in 2002 with the goal of ensuring and coordinating a high-quality laboratory service for measles and rubella diagnosis and surveillance. It comprises 71 laboratories, distributed through 49 of the 53 Member States of the Region. The GSL in London and three RRLs, sited in Berlin, Luxembourg and Moscow, supervise proficiency testing and assay implementation in national laboratories (NLs) and subnational laboratories.

As the European Region of WHO 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 measles/rubella RRLs was to update participants on progress towards achievement of previous global and regional meetings' recommendations, and on current status, issues and research. The participants also discussed present concerns regarding disease surveillance, laboratory verification, assay validation and training requirements.

This report summarizes 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.

2. Sessions of the meeting

Professor Maria Zambon, director of the Microbiology Reference Services of Public Health England (PHE) (Colindale, United Kingdom), opened the meeting welcoming the participants and describing the role of PHE in disease control, environmental and chemicals management. The creation of a National Infection Service in the near future and inclusion of some of PHE's services in its remit was described. It was highlighted that activities in PHE's Colindale site include not only virology work such as that in measles, rubella and influenza viruses, but also epidemiology and bioinformatics. She mentioned PHE's role in the control of the Ebola outbreak in western Africa, including the deployment of staff to carry out laboratory testing in Sierra Leone and control of incoming passengers at United Kingdom airports.

Numbers of measles cases in England have decreased since the large outbreak that affected England and Wales in 2012–2013. Current key areas of development at PHE include the use of whole genome sequencing (WGS) through next generation sequencing (NGS) methods as a potential tool to replace some of the existent assays.

The participants were invited to visit the laboratories at PHE and were wished a successful meeting.

Session 1 – Global and regional updates

Chair: Dr Kevin Brown

1.1. WHO European Region update on MR elimination programme

Dr Dragan Jankovic (WHO Regional Office for Europe)

Coverage with the first dose of measles-containing vaccine (MCV1) reached 94% in 2013, with most countries achieving 94–95% vaccine coverage at the national level. However, the challenge remains in achieving this coverage level in subpopulations. After a 98% reduction in measles incidence between

1993 and 2007, there has been a trend to a slight increase in measles incidence since 2011. Rubella incidence has remained consistently lower than that for measles.

According to 2014 data from the Centralized Information System for Infectious Diseases (CISID), there were 3248 cases of measles virus, although changes in the reporting system mean that the true number of cases may be up to 4690. Most measles cases (41%) have occurred in individuals older than 20 years, which are also the age group most likely to present unknown vaccination status – 90% of cases reported with unknown status are associated with this age group. Another group that raises concerns is health care workers, who were found to represent a significant fraction of all cases observed in several outbreaks in 2014: 40% in Czech Republic, 42% in Latvia and 25% in Spain. There are measles outbreaks presently being reported in Bosnia and Herzegovina (~6000 cases), Kyrgyzstan (~3000 cases) and Kazakhstan (>300 cases). Supplemental immunization activities (SIA) are ongoing in Azerbaijan, Georgia, Kazakhstan, Turkey and United Kingdom in response to outbreaks.

Rubella incidence decreased 98% between 2000 and 2011. Poland is currently the main source of concern, with 39 562 cases reported in 2013. The major issue there is historical, with no supplemental immunization activities (SIA) targeting the male population.

The action plan in 2015 will focus on improving the verification process for MR elimination and communication with regional laboratories in accordance with the Package for Accelerated Action of 2013–2015 and the European Vaccine Action Plan 2015–2020.

Countries will be grouped according to current achievements in the context of MR elimination. Each group will receive targeted support from the WHO Regional Office for Europe in terms of verification and capacity-building. Risks and better performers will be highlighted in order to increase each country's motivation to improve or maintain its status. Preliminary categories for measles and rubella elimination and countries included in each group were presented during the meeting. RVC conclusions regarding Member States measles and rubella status based on 2013 reporting are available in the 2014 RVC report (http://www.euro.who.int/en/health-topics/disease-prevention/vaccines-and-immunization/publications/2015/third-meeting-of-the-european-regional-verification-commission-for-measles-and-rubella-elimination-rvc)

In order to achieve MR elimination, shortages of communication in the past must be recognized, the partnership landscape must be optimized and graded and consistent messaging is necessary to maximize impact.

1.2. Update on implementation of 9th RRL meeting's recommendations, recent activities and 2015 planning

Dr Myriam Ben Mamou (WHO Regional Office for Europe)

Dr Myriam Ben Mamou gave an update on progress towards accomplishment of the recommendations from the 9th RRL meeting, recent activities and issues found by the WHO Regional Office for Europe and plan of actions for 2015.

In order to strengthen case-based reporting, an upgrade to MRLDMS is ongoing and the Regional Office is advocating for the use of case-unique identifiers at the national level. Standards accreditation and technical issues raised are being addressed to improve reference laboratories' compliance with WHO Labnet: enhancement of capacity in the processing of molecular data, increased detail provided

in confirmatory testing and assessment of FTA® cards as a tool to overcome cold chain/ customs issues. To enhance molecular surveillance of measles and rubella viral sequences and scale up reporting to MeaNS and RubeNS databases, GSLs and the Regional Office should provide training and feedback on the timely reporting of sequences to national reference laboratories (NRLs).

The Regional Office has conducted accreditation visits to Bosnia and Herzegovina, Tajikistan and Turkey and joint epi-lab country visits to Bosnia and Herzegovina and the Russian Federation. Additionally, the Regional Office has contributed to the Regional verification process, finalized the laboratory accreditation process for 2015 and overseen an upgrade of MRLDMS.

In 2014, only 22% of laboratories reported results within 4 days. Approximately 5% of laboratories received fewer than 50 samples/year and ~1.5% had less than 90% accuracy in IgM results. Performance was less satisfactory in internal quality control (IQC) procedures, with 65% of laboratories only partially following these. 56 laboratories (85%) do not have genotype data, do not report it or do not report it timely. The major concerns in laboratory procedures were biosafety (recurrent), the lack of reporting to WHO, the gaps in linking laboratory and epidemiological data and the lack of sensitivity of MR surveillance systems.

The country groups of London and Berlin are the ones reporting sequences from most laboratories, followed by Luxembourg and Moscow. In the context of verification, sufficient sequence data and links between epidemiological and molecular data will be necessary; hence the capacity of the laboratories to provide this information needs to be strengthened.

In 2015, the Regional Office will be rolling out the new MRLDMS, contributing to accreditation visits and promoting and facilitating communication and information exchange between laboratories.

1.3. Brief update on the implementation of 12th GMRLN meeting recommendations

Dr Mick Mulders (WHO headquarters)

The participants were informed on the progress made towards the implementation of the recommendations agreed during the 12th global measles/rubella laboratories meeting.

A working group to evaluate seroprevalence studies and laboratory methods for measuring measles and rubella antibodies is yet to be established, as global guidelines have not been finalized. Alternative methods for evaluating seroprevalence are being assessed, including a multiplex immunoassay (Luminex™), high-throughput neutralization assays and point-of-care tests. The re-evaluation of currently available IgM and IgG assays is being led by Dr Kevin Brown; the terms of reference must be developed and a working group defined.

A batch upload tool is being developed to facilitate timely and complete submission of all sequence data to MeaNS and RubeNS. This function will be rolled out to users of the databases. An algorithm is currently finding strains in the submitted sequences that can be used as named strains, which should be used in the description of outbreaks. Tools to support MeaNS and RubeNS data reporting to NVCs and the RVC are also being developed. Isolation of viruses is to be continued. A work group to assess the usefulness of expanding the measles virus sequencing window will be led by Dr Alberto Severini. Its members are to be defined and its terms of reference circulated. A vaccine-specific PCR protocol is to be developed at the Public Health Agency of Canada (RRL of the Pan-American Health Organization) and led by Dr Alberto Severini, but a deadline for the project has not been decided so far.

In order to improve surveillance, detailed epidemiological data should be provided in conjunction with laboratory results. The laboratory manual is currently being reviewed: a work group has been established and Dr Marilda Siquiera has the lead. A consultant is to be appointed and terms of reference defined and circulated. A draft protocol for the use of FTA® cards in the transport of clinical measles and rubella specimens has been developed by Professor Annette Mankertz.

A revision of the scoring for the serology proficiency test panel is underway and was discussed further in the meeting (see sections 4.3 and 5.2). Molecular EQA panels will be developed by INSTAND in Germany for European laboratories (see section 4.2).

1.4. GSL update: United Kingdom

Dr Kevin Brown (GSL United Kingdom)

in April 2013, the Health Protection Agency became Public Health England (PHE), which is part of the Department of Health. Although Wales, Scotland and Northern Ireland are now served by different laboratories, they still report results through PHE. Urgent measles testing is now done in regional laboratories. A large review and restructuring is underway at PHE due to budgetary constraints. A new National Infections Service will be formed, with the potential advantage of bringing epidemiology and laboratory surveillance under the same service.

Measles cases increased in 2012–2013 due to a large measles outbreak in England and Wales. More measles cases were observed in the 11–20 year age group, owing to concerns raised in 1998 over the safety of the measles-mumps-rubella (MMR) vaccine, which led to a reduction in vaccine uptake. As a result of SIAs conducted during and following the outbreak, the United Kingdom is getting back on target with vaccine coverage: ~95% of 5-yearolds have now been given at least one dose of the MMR vaccine and almost 90% of children in the same age group have been fully vaccinated (two doses). Given a good uptake of the SIAs, a dramatic fall was observed in the number of measles cases by the end of 2013 and a mix of genotypes is being observed, as expected if they were the result of importations.

Few rubella samples are being received per month at PHE, with only one case of rubella confirmed last year. Unfortunately, the child died in the United States after moving without PHE being aware of this and hence the case was not reported as CRI.

During the measles outbreak of 2012–2013, local testing was introduced in Wales. However, buccal instead of oral fluid (OF) samples were collected and positive samples were sent out for genotyping to Dublin rather than PHE as recommended. There have also been issues in receiving retest samples from Northern Ireland despite several requests. PHE has since proceeded to a roll-out of the measles PCR assay to public health laboratories (PHL). The test was modified, the use of a cellular control omitted and different types of samples were used. The GSL produced a validation panel, which was passed by all laboratories and requested that all local testing should be accompanied by OF samples. Testing at local laboratories started in September 2014 and will be evaluated at 6 months.

There have been issues with MicroImmune measles assays, with increased numbers of equivocal and false positive results being observed. 90 samples from 2012 were retested and the results confirmed the previous observations. A selection of samples was sent to MicroImmune for further work. Rubella IgG MicroImmune kits supply has been unreliable, with no kits supplied in over a year. This is due to

issues with rubella antigen stability. Communication with the supplier suggested the problem will not be resolved soon. Immunization history is now being assessed using the measles IgG kit.

At the molecular level, a new triplex PCR has been developed for measles, which uses fast technology, speeding up sample testing. This assay can detect two different regions (CDC primers for nucleoprotein and PHE primers for haemagglutinin) of the measles virus genome (confirmatory) and includes beta-2-microglobulin as a sample quality control. Rubella and vaccine-specific primers and probes may be added to the assay in the future.

There has been a consistently low number of submissions to measles and rubella strain banks. Most isolates were submitted by the United Kingdom and some from CDC. The strain bank is not representative of the circulating strains, in particular of rubella, for which most isolates are from congenital rubella syndrome (CRS) cases.

Current issues and concerns relate to improving communication and collaboration with laboratories with respect to sample retesting and result confirmation, particularly with those in Wales, Scotland and Northern Ireland, where there is increasing political focus on devolution of power. MR testing and surveillance may also be affected by national austerity cuts and restructuring/reprioritization within PHE.

1.5. GSL update: United States/Measles

Dr Paul Rota (GSL United States)

Elimination of measles in the United States was achieved in 2000 and verified in 2012. All recent cases of measles have been the result of importations of the virus from endemic regions. Many cases have been observed in unvaccinated populations, and some cases of vaccine failure have been detected.

Provisional data from 2014 indicates that the number of cases was 640 across 25 states, the highest recorded since 1994. 89% of cases resulted from 23 outbreaks. A large outbreak in Ohio represented 60% of cases. This outbreak resulted from the importation of measles virus (genotype D9) by religious workers who had been building homes in the south of the Philippines. Overall, 91% of cases resulted from international travel of unvaccinated residents of the Untied States. All outbreak-associated strains were sequenced, with the major genotypes detected being B3, D9 and D8.

The GSL in the United States has provided support in tackling a multistate measles B3 virus outbreak in the Federated States of Micronesia that occurred from March to August 2014. Most cases (65% out of a total of 389) were adults and one death was registered. A mass vaccination campaign was launched targeting 6–49-year-olds and so far 70 000 vaccine doses have been delivered. Currently, a large outbreak (genotype B3, Harare strain) resulting from exposure to an infected individual in an amusement park in California is ongoing, with 68 cases reported across 11 states since the 1 January, mostly amongst unvaccinated individuals.

Four vaccine-preventable disease reference centres are carrying out measles testing and genotyping and checking with CDC before submission of sequences to MeaNS (no issues have been found so far). The distribution of the workload between state laboratories may have led to a slight reduction in turnaround time.

Genotypic analysis has revealed that the measles genotype H1 sequences found in United States cases match those submitted from Chinese laboratories and that the B3 lineage found in an outbreak in Washington is not identical to the B3 Harare strain that was exported from the Philippines to most WHO regions.

1.6. GSL update: United States/Rubella

Dr Joseph Icenogle (GSL United States)

A new high-throughput immunocolorimetric assay for determining rubella neutralizing antibody titres has been developed. The method has been published and has now been used in several studies, including in a study of 1974 sera collected from 685 patients at different time points following a 3rd dose of MMR vaccine and of 322 sera also tested by various ELISA/Immunoblot techniques (in collaboration with Drs Christelle Vauloup-Fellous and Liliane Grangeot-Keros from the National Rubella Laboratory in France). CDC is also collaborating with PHE in the curation and maintenance of the MeaNS and RubeNS databases, helping to implement web code changes and to plan for new functionalities. It was highlighted that the code image for CDC is only to be used by a few individuals. Some issues have been found with sharing developments between CDC and PHE, which have mostly been overcome.

The CDC is initiating a new project which intends to study the immunocytochemistry of CRS cases in order to identify which cells are infected in CRI cases. Results could inform development of more accurate tests. In CRS, infection occurs primarily in spindle fibroblasts (which form a network for organogenesis). The CDC rubella laboratory is looking to initiate a collaboration with other laboratories to assess the implementation of a new non-structural protein (NSP) detection window. A survey to assess challenges in rubella and CRS surveillance in countries that are now starting is being prepared. The survey will be carried out in three countries, one of them in the WHO African Region.

1.7. RRL Berlin update

Professor Annette Mankertz (RRL Berlin)

Professor Annette Mankertz shared the results of proficiency tests coordinated by the RRL in Berlin. Of the 18 countries under the supervision of the RRL, most results have been received and analysed, with the exception of Denmark (no communication), Italy, Lithuania and Norway (pending). While most laboratories performed well, there have been some issues with communication: some laboratories failed to report results and others to provide a complete explanation for mistakes when this was requested. Some disagreed with the assessment, but communicated this to another laboratory and not to the one that should be following up on the results.

The number of IgM-positive results reported from Austria, Czech Republic, Latvia, Romania and Slovenia may suggest measles circulation. Slovakia reported a relatively high number of rubella IgM-positive samples, which also raises concern. Denmark, Finland, Lithuania and Poland are yet to report numbers of measles and rubella samples tested in 2014.

FTA®cards are being widely used for sample transport, both for outbreak-associated patient samples and for positive controls for PCRs. The protocol was developed at the Robert Koch Institute (RKI) and no problems have been found. However, duplicate test requests (for samples for which the sequence is already available on MeaNS) have occurred and should be avoided.

The Berlin RRL has published/is preparing five papers on topics related to measles transmission, outbreaks and molecular epidemiology. A new paper studying measles transmission patterns across Europe is also planned and will require collaboration with other RRLs and the GSL. Laboratory-wise, a new online request form for sample collection kits is now available and the implementation of a measles RT-qPCR assay has been successful. The Children's KiGGS2 survey is ongoing. Its goal is to assess risk factors to various mental and physical health conditions, including measles and rubella. 25 000 children between 1 and 17 years old have been surveyed so far and 10 000 laboratory tests have been conducted, which should allow the identification of risk factors for being unprotected and to correlate antibody titres with vaccination data. The survey should be completed by 2016.

New guidelines have been published for the diagnosis of rubella and measles. A new strategy was implemented for assessing rubella test requirements, which is strongly based on vaccine cards. Before pregnancy, when there is evidence of full vaccination, protection is assumed and no further measures are required; vaccination is carried out if there is no evidence of vaccination, no vaccination card or lack of status information. During pregnancy, no further measures are required when there is evidence of full vaccination; when there is no evidence of vaccination, no vaccine card or the status is unclear, the anti-rubella IgG titre is determined. If there is evidence of protection, no further measures are required, while if the IgG test is negative or equivocal, the pregnant woman should avoid contact, her family is vaccinated and vaccination is given post-partum.

Currently, a measles outbreak is ongoing in Germany. It originated among asylum seekers in Berlin and spread to the general population. Over 400 cases have been reported since early October 2014. No vaccination strategy is in place in Germany for displaced groups and insurance companies are not prepared to offer vaccination. The measles virus sequences reported belong mostly to the D8 genotype (Rostow-on-Don strain).

1.8. RRL Luxembourg update

Dr Judith Hübschen (RRL Luxembourg)

Dr Judith Hübschen informed the participants that the overall institution hosting the Department of Immunology (RRL Luxembourg) has been recently renamed Luxembourg Institute of Health . She reported that 19 out of 27 laboratories supervised by the Luxembourg RRL completed the measles proficiency panel with a perfect score. Six laboratories had issues with the same sample (fungal contamination), two had minor discrepancies in their results (an equivocal result for a positive or negative sample) and one had a major discrepancy (a positive result for a negative sample). For the rubella proficiency test, results were submitted by 27 laboratories, of which 25 achieved full result concordance. Two laboratories tested fewer than the 20 samples provided (insufficient volume left) and one had a major result discrepancy (a negative result for a positive sample).

The number of laboratories using dried serum spots for shipment is increasing and helping to overcome transport issues. For the first time in two years, the laboratory in Banja Luka, Bosnia and Herzegovina, is sending samples. There have been some issues with dried blood spots sent in from Italy as there is insufficient blood left for testing. Overall, laboratories are complying with the new instructions regarding which samples should be sent in for confirmatory testing and what additional information should be provided (e.g., samples only from suspected cases, additional laboratory results/final case classification). Kosovo (in accordance with Security Council resolution 1244 (1999)

and several countries (e.g., Greece and Portugal) are mainly screening surveillance samples, receiving few suspected case samples.

The Luxembourg RRL is carrying out measles virus (MeV) genotyping, updating protocols and conducting research activities such as outbreak investigations (e.g. in collaboration with WHO Laos) and reporting, whole genome sequencing of MeV and B cell repertoire investigations. The laboratory will be relocated to Esch, where it will be more limited in space. Two audits are expected in 2015.

1.9. RRL Moscow update including 2014–2015 action plan for RUS-NIS laboratory strengthening

Dr Tamara Mamaeva (RRL Moscow)

The Moscow RRL supervises 10 national and 13 subnational laboratories, 10 of which are located in the Russian Federation. All laboratories participated in the proficiency testing in 2014 and all achieved perfect scores both in measles and rubella proficiency tests. The kits used routinely by the 10 subnational laboratories in the Russian Federation were Vector-Best for measles and Ekolab for rubella, both of Russian manufacture. Two of the laboratories in the newly independent states (NIS) also used these kits, while the remaining 11 laboratories used the Siemens kits for measles and rubella.

Compliance with confirmatory testing has increased to levels accepted by WHO, including from those countries where fewer samples are tested. The kits used for retesting were Vector-Best/Ekolab, Siemens and Euroimmun. Results from all national and subnational laboratories showed 100% concordance with those from the Moscow RRL and only Tajikistan submitted fewer than 50 samples for confirmatory testing. In total, 1662 samples were subject to confirmatory testing, with 84.4% of rubella and 39.1% of measles samples testing negative for IgM.

Euroimmun kits have been shown to be equivalent to Siemens, the latter only being used for confirmatory testing. The use of sera and dried serum blots was tested with these kits and a preliminary protocol was developed. A further in-house study is planned to improve dried serum spot processing.

Approximately 77% of samples tested for measles in the Russian Federation are IgM positive. A total of over 8000 samples was tested that had been initially diagnosed as measles, rubella or other rash illnesses, with measles being confirmed in 50% of cases. The majority of measles cases were diagnosed in southern Russian districts (65%) and Moscow (22%). From 2008, the incidence of measles has increased from 0.019 to 3.1 cases/100 000 population. The fraction of cases confirmed by laboratory testing has consistently remained above 90%. Incidence rates for rubella are very low, with few samples being tested: in 2014, 47 pregnant women were tested and only one was IgM positive.

The Moscow RRL and the subnational laboratory of St Petersburg organized a workshop on seromonitoring in 2014 with the support of the Regional Office. 12 laboratories participated and have successfully completed tests. Another workshop was also organized by the Moscow RRL on the diagnosis of measles and rubella in which 5 subnational (Russian Federation) and 2 national (Kazakhstan, Kyrgyzstan) laboratories took part. Eight control panels with different levels of specificity have been developed and are already in use, four panels with 16 samples each for new kit testing and four panels with 20 samples each for internal quality assessment. These have been developed for dried-serum spot samples and will be rolled out for routine use in all 23 laboratories in 2015.

A workshop on measles/rubella PCR and genotyping will be held in May 2015 for the national laboratories of Azerbaijan, Belarus, Kazakhstan and Tajikistan. Once staff has been trained and is competent in the methods, visits to the laboratories will be organized. Later in 2015, a NIS workshop will take place, in which leading epidemiologists are invited to take part. Finally, a joint meeting of clinicians, epidemiologists and virologists from all laboratories will take place in October–November 2015.

1.9.1. RRL Moscow update – genotyping

Dr Sergey Shulga (RRL Moscow)

The update of the Moscow RRL was completed by Dr Sergey Shulga with information on measles genotyping. The laboratory is currently experiencing a lack of staff capacity to deal with the high number of submissions, hence the data presented is incomplete, but should be complete for the next global MR meeting.

The main measles genotypes found are D4 (Manchester strain) and D8. Measles virus strains of genotype B3 (Harare) were also isolated, albeit rarely and mostly as a result of importations. No endemic transmission has been detected for genotype B3. 90% of all measles cases were reported from the southern districts of the Russian Federation, with most other regions reporting little or no measles incidence.

Session 2 – Laboratory contribution to the verification process and case-based surveillance

Chair: Professor Annette Mankertz

2.1. Highlights and lessons learnt from the 2014 regional verification process

Dr Dragan Jankovic (WHO Regional office for Europe)

In 2010, WHO requested the initiation of a verification process for the European Region in order to motivate innovation and implementation of verification procedures. Dr Dragan Jankovic gave an update on the issues and conclusions taken from the Regional verification process in 2014.

The verification process is driven by the proof of absence of disease. This must include evidence of absence of endemic transmission of measles and rubella supported by genotypic information in the presence of an effective surveillance system. Supporting lines of evidence include epidemiological data on measles, rubella and CRS, molecular epidemiology data of measles and rubella viruses, information on population immunity and a sustainable national immunization programme.

The analysis will be made on a case-by-case basis due to the variability of systems in place and the data available. Endemic and unknown cases should be taken into account when analysing data and the total cases reported should be used as an indicator of prevalence. Importantly, laboratory data needs to be supported by epidemiological data

Analysis of the data provided by the Annual Status Update 2013 showed that there are issues with the completeness of the data reported, interpretation of questions and data analysis and presentation. Ten countries did not have their reports reviewed by the RVC, including three that were asked to resubmit due to missing data. A review of laboratory surveillance data showed that many countries did not adequately document virus transmission pathways. Although some areas are currently

performing better, there is year-to-year variation. The need to update the verification process criteria means that comparison of performance data from this year to data from the previous year is challenging.

Not all countries have provided national plans of action. According to preliminary data, plans of action have expired in Armenia, Bulgaria and France, are under development in Germany, Norway, the Republic of Moldova and Slovenia and have not been reported for Albania, Andorra, Bosnia and Herzegovina, Czech Republic, Denmark, Estonia, Finland, Georgia, Greece, Hungary, Israel, Kyrgyzstan, Luxembourg, Malta, Monaco, Netherlands, Poland, San Marino and Turkmenistan. The Region's countries have been grouped according to progress towards MR elimination (see session 1.1), but this grouping may be altered as more data becomes available.

The situation in 2013 is similar to that in 2012. Overall, more countries submitted reports and this was done in a more timely manner. However, the sensitivity of many countries' surveillance systems remains a concern. A final report on the data collected will be circulated and countries will be given feedback. The annual reporting form is under review and countries will be solicited to attach their opinion on the questions when submitting the form.

2.2. Update on MRLDMS upgrade process and next steps

Mr Tom Beesley (WHO consultant)

The updated functionality of MRLDMS was demonstrated by Mr Tom Beesley via teleconference. The new import system will be flexible, allowing for the import of data both in single file and in batch mode. Specimens and tests can be imported simultaneously, with the import system allowing several tests to be associated with one specimen and vice-versa. The focus is on flexibility and user-friendliness: the user will be allowed to import records with partial information and will be informed of the status of the operations taking place as well as of errors occurred. To allow for variation in the metadata available for different laboratories, each user can customise fields, for example by adding new fields for extra data, which will be available for every specimen created.

Once the data is in the system, it can be sorted and searched using customizable fields for each user. Fields of data to export may also be selected and the file can be named as desired. Data can be grouped and imported in connection to outbreaks, which opens extra options, such as outbreak-specific fields for data search and analysis. Outbreak reports can then be produced, grouping data by selected fields (e.g., specimen receipt data) and using multiple identifiers for each site.

2.3. Measles and rubella surveillance and reporting to TESSy

Dr Robert Whittaker (ECDC)

The European Surveillance System (TESSy) collects data for all diseases under surveillance at ECDC. Data collection for measles and rubella was started by the EUVAC.NET in 1999 and 2002, respectively and later transferred to ECDC in 2011. The data collected consists only of the variables requested by the Centralized Information System for Infectious Diseases (CISID) and is submitted to TESSy on an automated case base by some laboratories or aggregate by others. Submission of information relative to older cases can be done at any time. The data is briefly checked before being sent to CISID. Most delays in data submission are adjusted within one month, but reminders are sent to countries that have not uploaded data.

The data is aggregated, shared with the Regional Office and reports to Member States are produced on measles and rubella incidence in Europe each quarter, providing enhanced information on surveillance, epidemiology and geographical spread. In months not covered by the quarterly reports, a brief update is made available online, including maps, tables and a summary of main developments. Reports on CRS are produced on an annual basis.

In 2014, 30 countries reported case-based data for measles, totalling 3616 cases (down from 10 537 in 2013). Cases in Germany and Italy accounted for 58.6% of the total. Nine Member States reported less than 1 case/million population, with six reporting no cases. Over 40% of cases reported between 2006 and 2013 occurred in individuals over 14 years old. Rubella was reported on a case base by 26 countries and aggregated by one country. 6110 cases were reported in 2014, with Poland accounting for 96.5% of the total. 21 Member States reported low incidence of rubella (<1 case/million population), with 13 of these reporting no rubella cases.

Currently, ECDC is working on a project known as The Surveillance Atlas of Infectious Diseases (http://ecdc.europa.eu/en/data-tools/atlas/Pages/atlas.aspx), a publically available web-based tool for easy access to European infectious disease surveillance data through the ECDC website. For measles and rubella this will mean a change in the way ECDC displays the surveillance data reported by Member States. Measles data will be available from 1999, and rubella from 2007, displayed through common indicators such as the number of cases reported, notification rate, vaccination coverage rates and the distribution of cases by age, gender and vaccination status. In addition, new indicators will also be available including the separation of cases into endemic and imported/import-related, the notification rate of discarded cases and the display of data on a subnational level. The measles and rubella Atlases are currently under development. Once launched they will replace the current ECDC monthly reports on measles and rubella.

2.4. Sentinel surveillance of rubella in pregnancy/CRS; coordination of Renarub

Dr Christelle Vauloup-Fellous (National Rubella Laboratory, France)

In France, rubella surveillance in pregnancy has been carried out since 1976 by Renarub and mandatory antenatal rubella IgG screening has been in place since 1992. In 2012, a new national centre for rubella that focuses on rubella infection in pregnant women and congenital rubella was nominated. From 2013, surveillance of congenital rubella in children up to 1 year old and reporting of imported cases (individuals who return infected from abroad) was added in the Renarub surveillance programme. In the context of clinical diagnosis, the patient's IgM titre is tested when no recent evidence is found for vaccination. During pregnancy, if no previous IgG-positive test result is recorded and there is no proof of two vaccine doses, the first rubella IgG test is carried out as soon as possible, normally before 12 weeks of gestation. If the result is positive and there is no evidence for clinical rubella, no further testing is done. When the test result is negative, a second test is conducted at 20 weeks of gestation. If the patient still tests IgG negative, vaccination is administered post-partum. However, if seroconversion has occurred, the national reference laboratory carries out immunoblot/IgM testing on the first sample and IgM/avidity testing on the second sample. In all French laboratories, all sera are stored for at least one year to allow backdated testing; In the NRL, they are stored for 10 years.

IgM testing is essential to confirm rubella given that the rarity with which the disease is found in France means that the symptoms are not easily identified by clinicians. Importantly, the same kits and

protocols should be used in each test due to variations found between assays and laboratories. Standardized serology tests are crucial for the distinction between primary rubella infection and vaccination. This is because it allows for direct comparison of antibody titres: while IgM titres decrease more rapidly after the peak following primary infection than that following vaccination, IgG avidity reaches a lower plateau post-vaccination than that reached after acute infection.

Renarub is based on active reporting by all public and private laboratories to the NRL every trimester. The notification criteria are IgM-positive tests in pregnant women, foetal blood in newborns and children under 1 year old and PCR-positive results in ante-foetal samples, newborns and children under 1 year old or products of pregnancy terminations. The NRL is involved in case classification, providing additional laboratory expertise (avidity, IgM/avidity kinetics and immunoblot) for excluded cases, maternal primary infection or reinfection, CRI and CRS.

Following case classification, the NRL reports to the National Institute for Health. However, there are delays in reporting given the need to wait for delivery to report related data: there can be more than one year between the case and the report. At present, there is a tendency toward fewer bigger laboratories carrying out rubella testing. More physicians and laboratories respect the recommendation of not carrying out rubella IgM testing in pregnancy. The number of rubella cases in pregnant women is very low, with 12 cases of acquired rubella in France and two imported cases in 2013. No cases of CRS have been found when there is proof of vaccination.

Despite there being no rubella surveillance system in place for the general population, samples submitted as suspected measles cases which are PCR-negative for measles are tested by rubella PCR. However, given that the current surveillance system covers solely data in pregnancy, it only allows for confirmation of CRS elimination.

Session 3 – Molecular diagnostics and epidemiology

Chair: Dr Sergey Shulga

3.1. Update MeaNS/RubeNS

Dr Richard Myers (GSL United Kingdom)

In order to facilitate sample submission, a bulk upload function will be made available to all users of the measles nucleotide surveillance database (MeaNS). This tool will first request the user's details and then allow the upload of a comma-separated values file (.csv; exported from Excel). Fields will be added to represent epidemiological and outbreak links. A template for data import should be used and can be specifically developed for automated upload. Prior to submission, the data is displayed for user verification. When the user submits the data, it is validated by the system and, if the upload is not accepted, the reason for rejection is given. The main issues foreseen will be with date format, WHO name format and in assessing whether samples are acceptable for submission. Reporting tools/report production will be linked to each user.

It has been agreed in the past that named strains should be used in the description of outbreaks. Currently, the system identifies a named strain from a cluster of more than 50 identical strains. Recently, six H1, three D8 and two D9 new named strains have been identified. So far, the number of named stains does not cause issues, but in the future samples that are no longer circulating may be removed from the list.

A new pipeline for the generation of phylogenetic trees will be created. The new trees will include colour-coded genotype references and named strains. Anyone who would like the older system to be maintained should say so. The MeaNS database will be migrated to new servers in the near future (probably March 2015). This should improve speed and resilience and allow the use of a newer/more optimized operating system. There will be some disruption to service, but this should be minimal.

The GenBank is the largest submitter of sequences to the rubella nucleotide surveillance system (RubeNS). The coverage is patchy as there are not many sequences submitted, which might reflect reduced rubella circulation. There are sequences available for many genotypes, but the genotype distribution appears to be skewed. The majority of sequences submitted in 2014 were genotype 2B.

New code and required changes to RubeNS have been identified, developed and implemented in collaboration with CDC, which also carries out database curation. The sample display fields have been changed: fields are being updated in order to be more transparent and explain what is required to users. Similarly, queries have been updated, now allowing for the viewing of unlimited records in a single screen. The sequences available for each sample are indicated in the record list.

A MeaNS and RubeNS user survey will be carried out to identify usage issues and features required and to assess customer satisfaction. The results will be reported in the global meeting.

3.2. Molecular epidemiology in the WHO European Region, 2014

Dr Richard Myers (GSL United Kingdom)

There are 20 691 measles sequences available in MeaNS so far. There has been a gradual increase in the number of samples submitted, which results from higher sequence numbers being submitted by more users. Last year, the highest number of sequences was submitted by the WHO Western Pacific Region. Overall, the most common sequences in MeaNS belong to genotypes D4, B3, D8 and H1. In 2014, the genotypes most frequently observed were B3, D8, D9 and H1. The genotype distribution in the WHO European Region reflects the global pattern, which could be a function of reporting in the Region.

There appears to be a reduction in the diversity of circulating measles strains. It might be worth investigating whether this may be a marker for assessing the success of elimination/eradication efforts worldwide. The lineages of D8 in circulation are variable, with multiple original strains still found in 2014. In contrast, there seem to be only B3/Harare strains and their derivative lineages circulating in terms of measles genotype B3. In Europe, these strains have been circulating in many countries for the past 5 to 6 years with little sequence variation detected. Entropy plots show that D8 N450 sequences are more variable than those of B3, which may suggest there is an unidentified source of D8 that is seeding various outbreaks. Further discussion indicated that in other regions (Easter Mediterranean and African Regions) B3 sequences are more diverse. It was also suggested that a study of variability in D4 sequences could provide some insight into MeV evolution, given the existence of a large progeny of the D4/Enfield strain, now no longer found.

3.3. Use of FTA® and other filter papers for specimen transport: the DRC study

Dr Paul Rota (GSL USA)

The participants were updated on the findings of the study by Dr Paul Rota conducted in the Democratic Republic of the Congo (DRC), with the goal of assessing the use of FTA® paper for specimen

transport. During 2014, samples were collected for viral detection from suspected cases of measles or rubella at several local clinics in the DRC. Four samples were collected for each patient: two throat swabs and two Oracol® swabs. One pair of samples comprising one of each swab type (one throat swab and one Oracol® swab) was sent to the National Laboratory (NL) by standard reverse cold chain, processed and stored at -70°C. The second pair of samples was eluted at the local clinic on the day of collection. The eluted samples were then spotted onto FTA® cards (200µl), which were dried and shipped to the NL at room temperature and stored at -20°C. Finally, all samples were shipped to CDC for PCR testing in a single batch.

848 specimens were collected from 212 patients and tested by measles real time RT-PCR. Genotyping was done on selected positive samples. Samples were excluded for rubella IgM-positive patients and for patients testing measles IgM-negative and PCR-negative for all four specimens. Analysis of preliminary results suggests that using FTA® reduces sensitivity by approximately 10%, which makes the use of FTA® cards a valid option in the absence of an effective cold chain.

Further discussion clarified that FTA® cards are treated to inactivate the virus, maintaining the RNA – this involves protein denaturation and makes these cards unsuitable for sera transport. The positive samples collected using FTA® cards show higher Ct values, corresponding to an approximate two-fold reduction in titre, which might be an issue with rubella samples. Cards prepared in the field were found to be of lower quality due to failures in following the protocol. FTA® cards can carry four samples and may be stable at -20°C, but should probably be processed within a couple of months.

3.4. Can PCR serve as a primary tool for case classification?

Group discussion facilitated by Dr Kevin Brown (GSL United Kingdom)

Dr Kevin Brown initiated the discussion by pointing out the concerns of basing measles diagnosis solely on PCR. PCR false positives may result from laboratory contamination or detection of vaccine strain in a patient that has been vaccinated. Both of these situations would be identified by sequencing. False negatives can be attributed to insensitive assays, inadequate sample type or date of collection. Other concerns relate to the fact that PCR is less well-established in some laboratories, there are a range of protocols in place and samples being used and a proficiency programme is not yet in place for molecular assays. These concerns were demonstrated in the EUVac questionnaire and panel testing of 2010, which highlighted the high range of PCR assays in place, the variation in their sensitivity and the failure in using IQCs by some laboratories.

The participants agreed than the use of PCR as the primary method for diagnosis will require stringent conditions in terms of sample collection, assays used, quality control, proficiency testing and reporting of results. OFs were suggested as a potential preferred type of sample as they could be used to confirm less clear results by serology. A window of seven days was suggested as the most adequate for measles PCR samples collection, although it would vary according to sample type (longer for urine samples). For rubella, this window may need to be narrower (e.g., three days). Internal cellular controls should be used to track sample and assay quality and a robust proficiency testing programme should be put in place. All deviations from the recommended conditions should be reported and may require further testing.

The restrictions to the use of PCR as the primary diagnosis method should be further discussed and agreed at the global level. They would be specific for measles or rubella and clearly indicated in the

updated laboratory manual. It was highlighted that serology is still considered the gold standard for diagnosis in the WHO recommendations. However, given an increased move towards molecular testing and the difficulty experienced by some laboratories in obtaining samples for serology, it is necessary to specify the conditions in which molecular tests may be acceptable for diagnosis.

3.5. NGS – extended window

3.5.1. Measles whole genome sequencing

Dr Alberto Severini (Public Health Agency, Canada)

Both the N450 and the H regions of the measles virus genome are now sequenced on a routine basis at the National Microbiology Laboratory in Canada. However, the combined information from these two sequences is still insufficient to distinguish between endemic measles transmission and importations of the virus.

80 whole measles genomes have been sequenced so far in order to identify additional targets that might provide better resolution than the N450 and H gene sequences, determine how much variation is needed to exclude direct transmission between two measles cases and develop a practical method for whole genome sequencing (WGS) directly from clinical specimens.

The untranslated region between the M and F genes (M/F UTR) contains enough variation to be a good surrogate for WGS. Using this region, the chain of transmission that started a D8/Frankfurt outbreak was deducted. All strains from the outbreak had similar N450 and H sequences, but variation in the M/F UTR helped to identify three different transmission events at the start of the outbreak. Four nucleotide differences appear to be sufficient to exclude direct transmission.

A practical method for measles WGS is currently being developed. So far WGS is carried out using the Illumina MiSeq platform, but the coverage of the genome is uneven and works best on tissue culture isolates. Ten PCR reactions are required to obtain sequences for samples with high viral titres and three extra reactions are needed for lower titre samples.

3.5.2. Whole genome sequencing of measles virus

Dr Ana Penedos (GSL United Kingdom)

A project to assess the expansion of the sequencing window for measles virus is also underway at PHE.

A large outbreak of measles occurred in 2012–2013 in England and Wales. The English and Welsh cases appear to be related, with a single nucleotide difference found in the N450 sequence. However, the question remains of how significant a single nucleotide change is and whether it gives sufficient information to distinguish multiple importation events from genetic drift resulting from viral evolution. 46 oral fluid samples and 4 tissue culture isolates associated with the outbreak were selected and sequenced using an amplicon-based enrichment method combined with next generation sequencing (NGS) and Sanger technology.

Most of the genome sequence was obtained for 42 of the selected samples, ranging in concentration from ~10 to ~10,000 genome copies/µl. Little or no correlation was found between viral titre and sequence completeness. NGS coverage was over 90% for approximately 75% of all samples selected. Preliminary phylogenetic analysis of the sequences obtained suggests that the noncoding region

between the M and F genes provides a degree of tree resolution comparable to that obtained from whole genome sequences analysis, and hence the most resolution in relation to length of the sequence to obtain and analyse. Phylogenetic analysis of both the M/F UTR and the WGS suggests that the outbreak may have been initiated by 2 separate importations of the virus and the single N450 nucleotide change may have resulted from genetic drift.

Comparing entropy plots (which reflect nucleotide variation at each genomic position) for all genomes available in GenBank (all genotypes) to that for the D8 strains studied, suggests that N450 and M/F UTR are the regions where most variability is observed across D8 and all remaining genotypes. The data indicates that extending the sequencing window for measles virus will provide further molecular epidemiology detail at a time when virus diversity is decreasing and more countries approach measles elimination.

Session 4 – Quality assurance and capacity building

Chair: Dr Claude Muller

4.1. Global molecular PT: survey results

Dr Paul Rota (GSL United States)

The EQA procedure for 2014 is now complete and only one laboratory requested a retest due to problems in the sequencing facility (sample identifiers mixed). The data was not collected in a standard manner (Ct values provided with no plate layout, FASTA or chromatogram files for sequence data, variability in assay specificity), which complicated analysis.

The major scoring criteria for the next EQAs will be the ability to successfully detect measles and rubella RNA by RT-PCR (endpoint or real time), produce the required amplicons for genotype analysis and perform sequencing and sequence analysis to correctly identify the viral genotype. The proficiency tests will be scored as pass, fail or retest for both measles and rubella. To pass the test, the laboratories must achieve all the following: correctly detect measles or rubella RNA (or negative reaction) in all of the samples, have no false positive results, include positive and negative controls on PCR reactions as adequate, correctly identify the measles or rubella genotypes in each positive sample and be able to amplify and sequence the entire sequencing windows for measles (N-450) and rubella (739nt).

The participants were surveyed as to the best EQA format to implement in following years. 18 out of 21 countries agreed that proficiency testing should be carried out annually. The testing will be applied to RRLs and potentially extended to selected national labs at different times. From reception of PT panels, it was agreed that the turnover should be up to two months (9/21 of the laboratories selected one month and 10/21 selected two). 11 out of 21 surveyed considered that four samples for the measles and four for the rubella panel were sufficient.

The majority of the surveyed agree that the report of results should include a picture of the agarose gel (including all controls for standard RT-PCR), raw chromatogram files, sequences as aligned files (e.g., FASTA), the phylogenetic tree obtained and screenshots of the amplification plots for real time RT-PCR. 13/21 of the surveyed considered that reporting results by email is appropriate. The panels could be produced regionally, as long as all laboratories test the same samples, and results should be reported to the distributing laboratory. 11 of 21 find that the current pass/fail score is sufficient. The

use of FTA® cards was found acceptable by 19 out of 21 surveyed, but which filter paper to use should be specified and potentially distributed to guarantee that no cross-contamination occurs.

4.2. Scaling up molecular PT in the WHO European Region – Where are we now?

Professor Annette Mankertz (RRL Berlin)

The Robert Koch Institute has selected and produced virus stocks for measles strains to be used in the pilot testing of FTA® cards. Although high virus loss is observed when using FTA® cards (most of it during extraction), this should not constitute a problem for high viral titre samples.

An agreement has been reached with INSTAND Target Value Laboratories for the production of FTA® card-based measles EQA panels. Every panel will be pre-characterized by INSTAND before distribution. The EQA schemes shipment will contain four different samples, with two vials/sample, one for testing and one for back-up. Every sample should be reconstituted with 1.1 ml of PCR-grade water. There will be two EQA schemes distributed per year starting in 2015, one in June and a second in November. EQA schemes for mumps and rubella are ready to be rolled out too.

Protocols should be exchanged between laboratories and the last panel should be retested in CDC, RKI, INSTAND and probably United Kingdom's GSL. Professor Zeichhardt should be invited to the global measles/rubella laboratories network (GMRLN) meeting, the expansion of the molecular EQA discussed and a website for reporting developed. Given the small team at INSTAND, they cannot carry out training, but would be interested in providing other EQA panels. However, the short number of staff also means that INSTAND cannot be involved in the processing of samples beyond PCR testing.

In the ensuing discussion, it was suggested that cellular controls should be included in the panels. It was clarified that the panels will become commercially available as the goal is that other laboratories' performance can be monitored.

4.3. Serology PT: Revision of scoring criteria

Group discussion facilitated by Dr Mick Mulders (WHO headquarters)

The measles/rubella IgM proficiency testing is done in order to assess the proficiency of laboratories in the WHO global network, identify issues with routine testing assays, verify accuracy of data reporting, assess the criteria for assay validation and check timeliness of result reporting (within 14 days of panel reception). However, the current scoring is mostly based on the results reported, not taking in consideration quality control and timeliness of reporting.

A proposal for a new scoring system where more points are associated with results, indication of reagent details and timeliness of the reporting was presented and the participants were asked to comment on the proposal. All participants agreed that the scoring must be rigorous and include criteria on validity of reagents used (e.g., use of expired or non-recommended kits should be penalized), run validation and timeliness of reporting. The kits used in proficiency testing should be the same as used in routine work. It was suggested that it would be relevant to look at calculations for result reporting to detect any mistakes in result interpretation. The timeliness of result reporting will be strictly assessed, with exceptions looked at on a case-by-case basis in outstanding circumstances that are out of the laboratory's control.

4.4. IgM confirmatory testing: update of instructions and revision of reporting form

Group discussion facilitated by Dr Judith Hübschen (RRL Luxembourg)

The participants were asked to give suggestions and comments on the update of the instructions relative to IgM confirmatory testing and whether the report form should be reviewed.

The discussion was centred on the fact that many surveillance systems are not receiving sufficient numbers of samples to send for confirmatory testing, particularly rubella samples. It was pointed out that if fewer than 50 samples are received for testing each year, the samples received should be topped up by samples received for investigation of any fever-rash diagnosis. Even if less than 50 samples, only samples from suspected cases should be sent. The minimum number of samples required for IgM confirmatory testing may be reviewed for countries with smaller populations. When it is impossible to satisfy the minimum number of confirmatory samples to submit, it may be demonstrated that cases of measles or rubella are still being detected when they occur.

In conclusion, it was agreed that the samples to be submitted for IgM confirmatory testing are, in order of preference: surveillance positive and negative samples from suspected measles or rubella cases and rash/fever illness surveillance samples (ideally positives). When the minimum number of samples (50 or more to fulfil the "at least 2 discarded cases per 100 000 population" indicator) cannot be achieved, it should be explained why.

4.5. European Region MR Labnet training: needs and plans/e-training options

Group discussion facilitated by Dr Myriam Ben Mamou (WHO Regional Office for Europe) and Dr Joseph Icenogle (GSL United States)

Dr Myriam Ben Mamou explained that a training needs assessment is required to inform the delineation of a training plan for the European Region Labnet laboratories. The assessment would help identify gaps and how to address them, to ensure that the relevant training is delivered and the outcomes are evaluated so that the use of resources is maximized.

Existing sources such as accreditation checklists, genetic databases, RRL knowledge of associated laboratories, results of on-site accreditation visits and self-assessments by NRLs can be used as sources of data for identifying the training needs. It is important to keep in mind that training may not be the answer for the issues found or may need to be used in conjunction with other approaches. Non-performance may result from problems other than a lack of knowledge or skills, such as unsustainable funding, non-sensitive surveillance systems or lack of ownership or commitment.

Training may target laboratory methods, analysis of sequence data, data reporting (e.g., to MeaNS and RubeNS) and contribution to NVC reports. It could be administered through hands-on laboratory workshops, e-learning or webinars or individual training at the RRLs. Finally, the proposed training should be assessed in terms of benefits and effectiveness.

Dr Joseph Icenogle highlighted the advantage of video to clarify issues, both through video-conferences and webinars. E-learning courses and webinars can be accessed internationally and at the pace of the trainee. However, there are strict rules on the distribution of electronic materials and the preparation of these is lengthy and requires specific software available. It is important to keep in mind

when producing e-learning resources that lectures should be short in order to maintain attention and overcome the attention deficit found in non-face-to-face interactions.

In the discussion following both opening presentations, it was agreed that training needs will be more effectively identified by the Regional Office and RRLs than through laboratory surveys. The type of training should be targeted to the gap identified as well as to the trainee. While e-learning and teleconferences between laboratories may be appropriate to address some issues, face-to-face training will be the most effective at providing an understanding of the potential problems and how to troubleshoot. Workshops and courses are more effective if they include a follow-up with trainees. Training of staff through visits to the RRL was suggested as the best way of familiarizing trainees with assays, problems likely to be found and result analysis. The context and outcome of training activities should be recorded and used as an indicator of effectiveness of the activity for addressing specific training needs.

Session 5 – Serology

Chair: Dr Paul Rota

5.1. POCT update

Dr Dhan Samuel (PHE, United Kingdom)

A new point of care test (POCT) for measles IgM antibodies is being developed and improved at the Serological Development Unit in PHE. The objective is to provide a commercially available assay to detect measles infection in the field.

Currently, Oracol™ swabs are used to collect OF samples for serology and molecular testing. These swabs are rubbed on gum margins for approximately one minute, eluted with diluent and spun down to remove any diluent remaining in the foam. A similar sample collection kit is being developed that eliminates the need for centrifugation. The sample from the Oralight swab can be eluted mechanically by compressing the swab using a specially designed vessel.

This vessel is also designed to allow for the application of sample drops onto a test strip. The latter consists of a lateral flow device made of nitrocellulose membrane. 5 µl of rNP antigen and 100 µl of sample are added to the strip or to a tube in which one extremity of the strip is dipped. In this side of the strip, there are monoclonal anti-nucleoprotein gold conjugate antibodies to which only anti-measles IgM antibodies can bind (through the added rNP antigen), then followed by a test line with bound anti-human IgM antibodies for the detection of anti-measles virus IgM antibodies; and finally a control line with bound anti-mouse IgG antibodies allows for the detection of the monoclonal anti-nucleoprotein gold conjugate antibodies (indicating successful sample migration). An inexpensive lateral flow reader can be used to read test strips results and transfer them in real-time.

Oralight swabs are sufficiently different from Oracol™ swabs to require validation. This includes proving safety of the device (e.g., extractable and leachable, non-toxic, ethical approved) as well as assessing its performance (e.g., sample recovery, volume required, diluent required, possibility of nucleic acid recovery). The team are currently working with Abingdon Health (who took over Forsite) and, after some delays, the first test cassettes will be received by April 2015. Following initial testing

of these, a pilot lot of 3000 cassettes will be produced. The cost may be as low as £0.83 for the extraction device and between £1-2 for the strips.

5.2. ELISA comparative studies/Preliminary analysis of proficiency panel testing data

Group discussion facilitated by Dr Kevin Brown (GSL United Kingdom)

Concerns have been raised previously regarding observed differences between various assays; and the need to conduct a comparative study looking at the sensitivity and specificity of the different assays has been mentioned. However, before such study was considered, it would be necessary to identify the exact question(s) being addressed: would the kits be tested for the detection of infection, cases leading to onward transmission, re-infection or vaccine failure. The type of assay adequate to answer each of these questions will vary. For example, indirect ELISA is most widely used to assess seroprevalence: the signal is enhanced and the assay can be modified to measure avidity. However, these assays tend to have lower specificity, be subject to inhibition and measure the antibody as a proportion of the total antibodies to the antigen of interest. On the other hand, capture assays are very sensitive to lower antibody levels (e.g., OF samples) and measure specific IgM relative to total IgM, but are less appropriate for determining IgG levels.

A comparison of the proficiency panel results obtained with various kits between 2011 and 2013 was carried out. All data for which test cut-offs were incorrect or unclear or for which the value reported had not been specified (i.e. OD or T/co ratio) were discarded. For the Siemens assays, only data from laboratories which had corrected the OD were used. A test cut-off value was calculated for all results and a cut-off of 0.15 was used for Siemens assays to allow for the equivocal range. A “discriminatory index”, representing the quotient between the mean of positive and negative samples, was then calculated for each kit/year. Siemens is the most used assay and performs consistently well, achieving a high discriminatory index. No problems are found with other assays used either.

The participants consider that a workgroup should be established to assess which questions are to be answered (e.g., primary or secondary infection diagnosis) and if using the proficiency panel data and INSTAND’s database might provide them. Guidance on which kits are most adequate to answer a specific question or for different sample types/collection times should be included in the laboratory manual, as well as the limitations of specific assays for addressing some questions. Likewise, assays that are performing poorly should be identified. Issues for measles and rubella differ and should be indicated.

If it is decided that a comparative study is necessary, several kits should be tested by selected laboratories. Panels should contain a well-defined set of specimens, including measles, rubella and dengue (for instance) to address cross-reactivity and the set of specimens should be well defined. The sera to test in such a study should be well characterized and results should be confirmed by PCR.

5.3. Seroprevalence studies

Group discussion facilitated by Dr Christelle Vauloup-Fellous (National Rubella Laboratory, France)

WHO must provide guidance and support in the implementation and assessment of seroprevalence studies. The participants discussed issues, advice and limitations in conducting seroprevalence studies.

It was pointed out that seroprevalence studies may be less informative than they are believed to be. In regions where the cold chain is reliable and there is an effective surveillance system in place,

seroprevalence studies are of limited interest. When this is not the case, there may be issues with variability of the results obtained and vaccination information may be more informative. For instance, in regions where vaccination coverage is good and there is little virus circulation, seroprevalence can be very low.

To proceed to a seroprevalence study, the question being asked must be defined and the assay to use should be decided on that basis (e.g., ELISA IgG can be used to address immunity), taking into consideration the population and disease being studied and limitations of the assay. Clear guidance must be issued in terms of type of samples to collect and results interpretation as they vary with the kit used (e.g., different cut-offs for different kits). If assessing seroconversion after vaccination, pre- and post-vaccination sera need to be tested in parallel. Seroprevalence studies have limitations in providing guidance for vaccination as the results obtained are variable and not straightforward to interpret. Furthermore, these studies may be lengthy: results may thus reflect a point in the past, become available too late (e.g., when an outbreak has already started) or lead to erroneous vaccination guidance.

5.4. Planning European Region MR Labnet upcoming publications and contribution to global MR Labnet publications

Group discussion facilitated by Dr Mick Mulders (WHO headquarters)

The strategy for the publication of papers by the Labnet laboratories was discussed. The participants agreed that publishing in a scientific journal is more motivating than using WHO publications. However this requires richer content and more in-depth analysis. Given that reviewers tend to reject papers analysing single outbreaks, it may be a role for RRLs to coordinate the publication of multi-outbreak studies with general conclusions from the collection of data or to approach publishers suggesting a special issue, although coordinated submission of papers may be difficult. More depth can also be added by conducting meta-analysis of the data rather than using solely sequence information.

A study describing patterns of measles transmission in the European Region has been proposed by the RRL of Berlin and collaboration with other laboratories will be needed to provide a general picture of the Region. The Moscow RRL is working on a paper to describe the work carried out in serology for over 11 years. Initially it will be published in Russian journals, but it was suggested that it would be worth publishing in English as well. The GSL in the United State has a manuscript in progress looking at fatal cases of CRS. The study is an update to the last paper published on the subject in 1967 and investigates which cell types are infected in fatal CRS cases using fluorescent immunocytochemistry.

3. Recommendations

The following recommendations were agreed on by the participants in exchanges and discussions during the meeting.

3.1. Accreditation

1. Proficiency testing panel results: WHO Labnet should apply a more stringent scoring system to PT results. VIDRL is requested to test several proposals against current 01404 PT, to share for discussion and agreement on the final scoring system to be applied in the next round.
2. Retesting: In order for the 2014 recommendation to be fully implemented, the RLC should circulate the revised retesting form as well as the updated instructions for harmonization

between RRLs in the European Region. RRLs agreed on getting samples for retesting from suspect cases only, even if the number is less than 20 (see corresponding recommendation from 2014 RRL meeting).

3. Molecular PT: it is recommended to roll it out for all NRLs providing independent results for molecular testing (WHO, CDC, RRL Berlin)
4. MRLDMS has been upgraded with additional functionalities: RLC to expedite finalization and RRLs to collaborate for piloting.

3.2. ELISA comparative studies

5. It is recommended that a working group be set up to further discuss and agree on the principle of conducting ELISA comparative studies, and define future steps regarding the design and implementation of these studies (GSLs, RRLs, WHO).
6. If the decision to conduct ELISA comparative studies is confirmed, the panel and protocol should be well-defined and questions to be answered should be agreed on (ELISA studies working group).
7. It is recommended that the upcoming revision of the *Measles and Rubella laboratory manual* be taken as an opportunity to integrate a section providing comprehensive guidance on kit selection (Manual revision working group).
8. Informative data from kit comparison have been made available from PT panels. It is recommended that this data be summarized and disseminated (GSL London, WHO headquarters).

3.3. Molecular detection/surveillance

9. PCR as an exclusive tool for measles cases classification should be a possible option for NRLs (serology stays as the first option). Comprehensive guidance in the lab manual for quality PCR is requested: which samples, time of collection, IC procedures, molecular PT (Manual revision working group).
10. In order to generate more genotyping data, countries are encouraged to use FTA® when appropriate.
11. WGS results are promising. GSLs and RRLs are encouraged to continue investing and exploring this tool in their research and development activities. However, this technology is not recommended for wide use in the European Region now.

3.4. Verification of elimination

12. Reference laboratories are urged to increase timeliness and completeness of reporting to WHO nucleotide surveillance databases MeaNS and RubeNS: all measles and rubella sequences generated should be submitted in a timely manner.
13. Provide appropriate guidance to NVCs for the development of country Annual Status Updates: increase integration of epidemiological data with sequence information to inform RVC on virus transmission pathways (Regional Verification Commission, WHO Secretariat).

14. Seroprevalence studies: Circulate the draft of WHO Global guidelines on seroprevalence studies among RRL meeting participants for comments, labnet to provide expert guidance on how to conduct quality seroprevalence studies (seroprevalence/immunity, assays, samples).

3.5. Capacity building/training

15. Opportunities should be provided for laboratory training and capacity building. Training approaches and content should be tailored to training needs, to be identified from the performance perspective (WHO, RRLs, GSLs).
16. It is recommended that existing mechanisms and sources of information be used to assess training needs: accreditation check-lists and on-site visits, verification process and documents, GSL /RRL expertise, environment analysis (WHO, RRLs, GSLs).
17. When developing trainings, organizers are recommended to ensure coordination and harmonization and make use of already existing resources, including e-learning options available from CDC labs (WHO, RRLs, GSLs).

3.6. Publications

18. Global MR Labnet is strongly encouraged to publish in upcoming issues of Morbidity and Mortality Weekly Report/Weekly Epidemiological Record, a comprehensive update on measles genotyping, including guidance about strategy of genotyping during outbreaks, and approaches to identify separate clusters (WHO, GSLs).
19. European Labnet is strongly encouraged to publish papers on the specific features of the Region in the context of elimination: progress and issues of the Region (chains of transmission, genotype replacement). Additionally, regional publications with innovative ideas or new perspectives on existing data are welcome (RRLs, GSLs).
20. Seroprevalence studies from Russian Federation have been published earlier in Russian. Publication of these data in English journals will be highly appreciated, as this would allow wider dissemination and use (RRL Moscow).
21. There is a need to publish a paper on MeaNS. A proposal should be presented (to MeaNS/Rubens Steering Committee) at the global MR Labnet meeting in June 2015 (GSL, WHO).

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: contact@euro.who.int // Website: www.euro.who.int