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WHO meeting on strengthening the measles and rubella laboratory network in the Russian Federation and newly independent states

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Abbreviations

EIA	enzyme immunoassay
FTA	Fast Technology Analysis
MeaNS	Measles Nucleotide Surveillance database
MR Labnet	measles and rubella laboratory network
MRLDMS	measles and rubella laboratory data management system
RubeNS	Rubella Nucleotide Surveillance database
NIS	newly independent states
NRL	national reference laboratory
RRL	regional reference laboratory
SNL	subnational laboratory

Introduction

This WHO meeting on strengthening the measles and rubella laboratory network (MR Labnet) in the Russian Federation and newly independent states (NIS) (20–22 May 2015) was a follow-up of the meeting on the updated verification process and measles and rubella elimination requirements in the WHO European Region (19–20 May 2015), which hosted, along with chairpersons of the national committees for verification and epidemiologists, also the heads of the national laboratories, as strongly recommended by the participants at the 2014 Russian Federation-NIS MR Labnet meeting.

The participants of the meeting on the updated verification process have repeatedly underlined the utmost importance of the laboratory data for verification of measles and rubella elimination. The format of group sessions provided a platform for the heads of laboratories to discuss jointly with epidemiologists the updated national template for the measles and rubella elimination status report.

The meeting on strengthening MR Labnet in the Russian Federation and NIS was attended by the heads and representatives of national and subnational measles and rubella laboratories (NRL and SNL respectively) from 10 countries (Armenia, Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan, Republic of Moldova, the Russian Federation, Tajikistan, Turkmenistan, Uzbekistan), employees of the Regional Measles/Rubella Reference Laboratory in Moscow, employees of the Global Measles/Rubella Reference Laboratory in London and representatives of the WHO Regional Office for Europe (Regional Office).

Sessions of the meeting

Session 1 – Regional update

After reviewing the recommendations from the 2014 meeting, Dr M. Ben Mamou briefed the participants on the current status and performance of the European MR Labnet. Approximately 40 000 specimens were examined in the Region in 2014 and over 55 000 tests for measles and rubella were run. At the same time, the workload of the laboratories differed significantly. The measles genotyping results, placed in the Measles Nucleotide Surveillance (MeaNS) database, testify dominance of D8 genotype and a decreasing role of D4 genotype in the Region. The number of registered rubella cases is still small, which accounts for limited data that is channeled into the Rubella Nucleotide Surveillance (RubeNS) database, with only 67 sequences submitted by eight countries of the European Region over the past five years. The 2B genotype rubella virus is the most widespread. In the NIS subregion, only four countries have information on measles virus genotypes for the past year, and no country on rubella virus; this area of activity must be improved. By the end of 2014, all 11 NRL and 12 SNL in the NIS received accreditation. 100% completed laboratory reports, however, the reports were not always submitted in due time. Moreover, internal quality control procedures are not fully implemented and require further attention. Apart from that, the main challenges are as follows:

- to strengthen commitment and contribution of the countries in achieving the elimination goals;
- to improve linkage between epidemiological and laboratory data;
- to provide for genotyping of at least 80% of virus transmission chains;
- to provide laboratory confirmation of rubella and causative agent's genotyping data;
- to submit lab results into the surveillance system within four days;
- to timely channel the information into all WHO databases;
- to ensure timely sending of the specimens and uninterrupted performance of the laboratory network, including complete sequence data reporting to MeaNS and RubeNS.

Dr T.A. Mamayeva spoke on behalf of the regional reference laboratory (RRL) of the Russian Federation and presented data on laboratory support in implementing the measles and rubella elimination programme in NIS. All the laboratories of the subregion demonstrated high-quality performance in 2014. The countries run IgM identification test-kits of various manufacturers, namely: *Euroimmun, Siemens and VectorBest*. This notwithstanding, testing of all 1639 samples in the RRL showed 100% match for both measles and rubella results. Armenia, Turkmenistan and Ukraine have successfully delivered specimens for re-testing in the form of dry serum drop; this best practice could be followed by other countries. All 23 participating countries have successfully run the professional testing. With the exception of the Tajikistan laboratory, which recommenced activity as recently as the second half of 2014, all the laboratories ran enzyme immunoassay (EIA) for over 50 specimens per year. However, implementation of the internal quality control needs to be facilitated; less than half of the labs use it today. The RRF has introduced intra-laboratory control samples, which shall be further extended to the whole network. The seminars on serological studies organized by the RRF with support of the Regional Office helped to optimize laboratory performance. Dr Mamayeva suggested using the EIA "capture" option to detect IgM antibodies in vaccinated patients since it has higher sensitivity compared to the "indirect" EIA option. The following challenges have been recognized:

- Reports to RRL should be submitted in a timely manner.

- Samples for re-testing should be delivered at least 2 times per year.
- Cooperation with epidemiologists and clinicians should be consolidated.

During the discussion round, it was specifically stressed that detection of IgM should remain the basic method for confirming the diagnosis, while polymerase chain reaction (PCR) should be seen not as an alternative thereto, but rather a supplementary method.

Dr S.V. Shulga shared the data on molecular epidemiology of measles and rubella in the Russian Federation and NIS. All in all, measles viruses of 15 genetic variants of D4, D8 and B3 genotypes were identified in the subregion in 2013–2014. Just like in the whole European Region, D8 remains dominant. In recent years, the biggest measles outbreak in the subregion was recorded in Georgia and was caused by D8 Frankfurt. Viruses of D4 and D8 genotype spread in Ukraine. Measles viruses detected in 2014 in Belarus, Kazakhstan, Kyrgyzstan and Uzbekistan also belonged to D8 genotype. A dramatic change of the dominating genotype from D4 to D8 was recorded in the Russian Federation after 2012. Eight and six genetic variants of D8 genotype circulated in the country in 2013 and 2014 respectively. Such genetic variety is the evidence of multiple importations of the virus. None of the country's regions saw circulation of any variant for over 12 months; in other words, endemic circulation has not been restored. Genetic information for rubella for the period of 2013–2014 is available for 13 strains, 12 of which belong to the 2B genotype and one to 1E. Not a single H1 genotype strain, endemic for the subregion prior to 2011, has been identified. Rubella outbreaks of 2011–2014 are connected with importation cases and limited local circulation of 2B and 1E genotype virus strains of "Asian" origin. Both genotypes are represented by several strain clusters, which is indicative of their independent origin. Dr Shulga stressed that while genotype circulation is becoming globalized, investigation of each separate case is of increasing importance. The main objective is defined as follows: to ensure maximum identification of imported cases and to establish duration of the virus genetic variants circulation.

Dr Kevin Brown from the London global specialized reference-laboratory gave a presentation on updated measles and rubella serology. He pointed out that serology today implies testing not only blood serum, but all the biological liquids as well, and the choice of the best possible method depends on the type of the specimen, and the specific purpose of the study. The report highlighted advantages and weaknesses of a number of EIA options for identification of IgM and IgG antibodies. The "capture" option was mentioned as the most sensitive both for studies in cases of re-infection and for testing of the oral liquids. Dr Brown shared with the audience the possibilities of multiplex testing using western-blot, multiple conjugates and luminex technology. Oral liquid was referred to as a good alternative to blood serum. This specimen is non-invasive, it matches serum in terms of sensitivity and specificity and can be used for identification of both IgM and IgG, and for PCR purposes. With regard to sero epidemiological studies, Dr Brown underlined that threshold indicators for antibodies in the population may not match the threshold numbers given by the manufacturer, and would ask for specification in case such studies are conducted. Application of international standards is essential in ensuring quality control; however, these standards should only be applied with regards to those formats of the tests for which they have been calibrated. The presentation was received with much interest by the participants. The consequent discussions focused mostly on standardization of methods, use of field tests and availability of test-systems for examination of oral liquids.

Session 2 – Updates from the national and subnational laboratories

Measles and rubella surveillance results in the Region were presented in the reports of 20 laboratories, among which: national laboratories of Armenia, Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan (and one subnational), the Republic of Moldova, Tajikistan, Turkmenistan, Uzbekistan and 10 subnational laboratories of the Russian Federation.

All the reports followed the WHO-suggested format. The highlighted strengths of the laboratory performance were as follows: results are submitted to the surveillance system in a timely manner; WHO criteria are observed; external quality control has scored good results; the laboratories received accreditation by the relevant national authorities; national programmes for external quality assessment have been set up for regional laboratories (Kazakhstan); Russian Federation provided equipment for molecular studies.

The major challenges in the activity of the laboratories are connected with untimely delivery of specimens to be tested in the country and difficulties with their dispatch for re-testing in RRL, low quality of the specimens received for genotyping, particularly intense workload (the Russian Federation), outdated equipment for EIA and high staff turnover (Kyrgyzstan), lack of cooperation with private laboratories (Republic of Moldova), inconsistency between laboratory and epidemiological data, absence of the basic funding and problems with procurement of consumables.

Many laboratories referred to introduction of molecular and genetic methods of studying measles and rubella, training of specialists in molecular methods, introduction of dry blood drop method (Kyrgyzstan) and ensuring timely reporting to the regional laboratory and WHO as future perspectives.

Training on WHO sequence surveillance databases

As recommended during the previous Labnet meeting in 2014, the third day of the meeting focused on **training the participants on WHO measles and rubella genetic databases**. Dr Richard Myers shared information on principles of organization and functioning of databases on nucleotide sequences of measles and rubella viruses – MeaNS and RubeNS, respectively. He demonstrated to the participants the procedure of giving a unique WHO name to a strain, the possibilities for use of the databases, and levels of permits the users can benefit from and how this database is connected with GenBank.

Next, the audience was presented with step-by-step instructions for registration in a database, placement therein and analysis of nucleotide sequences. During the subsequent practical exercises, guided by Dr Myers and Dr Brown, all the participants were able to go through the registration process and received access to the databases. While practicing, the participants acquired the following skills: how to give a name to a strain and introduce sequencing data; how to search for the data and sort it out; how to conduct sequences analysis and genotyping using reference-sequences. Everyone successfully completed the provided tasks. In conclusion, Dr Myers dwelled on data analysis using MeaNS and RubeNS databases, including distribution of all the available sequences by genotypes, countries and WHO regions. The training course sparked a lot of interest among the participants and everyone completed the practical tasks. The participants agreed that the acquired knowledge helped them to better understand the importance of submitting the information to the WHO nucleotide databases in a timely and complete manner, thus allowing for global assessment of how the circulating genotypes are spread and using this assessment for the purposes of molecular epidemiology.

Recommendations

Based on the provided data, the meeting highly valued the performance of the measles/rubella subregional laboratory network in 2014–2015 (4 months) and deems it necessary to highlight the following.

Political commitment, responsibility and partnership

The meeting on the updated verification process in the WHO European Region, which along with chairpersons of the national committees for certification and representatives of epidemiological services hosted also the heads of the national laboratories, highlighted the utmost importance of the laboratory data for verification of measles and rubella elimination.

1. The countries' commitment to implement the measles and rubella elimination programme is crucial for success. Considering the principal importance of the laboratory data at the stage of disease elimination, it is deemed necessary to increase the countries' contribution to supporting national reference laboratories in order to ensure their uninterrupted and sustainable performance. Should there be a need to extend measles and rubella diagnostic studies, the countries are encouraged, to individually procure RRL recommended test-kits, along with WHO supplies.

2. The countries' support is needed to organize transportation of specimens both within the country to the reference laboratories and from the reference laboratories to the RRL for confirmation. In this connection, facilitation of customs clearance procedures is welcomed.

3. Supply of equipment, carried out by the Russian Federation to the NIS laboratories is acknowledged as a valuable contribution to supporting the implementation of the measles and rubella elimination programme in the WHO European Region. Strengthening the material base of the laboratories should be conducive to improving the quality of the studies and expanding the amount of molecular data submitted to the programme. The Regional Office welcomes activity of the RRL in Moscow targeted on training NRL employees to run molecular-genetic study of measles and rubella viruses.

Improved integration of laboratory and epidemiological data and improved case-based surveillance to ensure infection elimination verification

Case-based surveillance with laboratory confirmation of the diagnosis is of key importance for monitoring the progress in implementing the measles and rubella elimination programme. A clear linkage between epidemiological and laboratory data plays a crucial role in timely classification of cases, detection of outbreaks, development of response measures, assessment of the elimination status in the country and in general, in taking well-informed decisions.

4. The effective surveillance system over measles, rubella and congenital rubella syndrome is the key prerequisite for a successful measles and rubella elimination programme. It is

recommended to all the countries to give special consideration to congenital rubella syndrome surveillance, in particular, wider case detection and laboratory investigation of suspected cases.

5. The countries should seek full compliance between laboratory and epidemiological data and regularly analyse the results of all surveillance components at the national level. Introduction of an individual case identification number at the national level will facilitate improved data collection and analysis. Close cooperation of epidemiological and laboratory service will allow for optimizing selection of specimens for genotyping, improving documentation of transmission chains and efficiently using molecular data for epidemiological analysis of the situation.

6. The meeting requests the Regional Office to carry out training on using the online measles and rubella laboratory data management system (MRLDMS). WHO welcomes implementation of this system in all the countries of the subregion for filing reports to the WHO. Use of this online system will enable case data submitted by a country/region to be updated with genotyping data received from the RRL.

7. Besides the three essential criteria to verify measles and rubella elimination (absence of endemic measles and rubella cases, the presence of a high-quality surveillance system and genotyping evidence), data originating from quality seroprevalence studies may also be considered as supplementary information to identify immunization gaps and susceptible groups with increased risk of infection circulation. If seroprevalence studies are performed, countries are recommended to follow WHO regional and global guidelines, and pay careful attention to select an adequate study design and laboratory tools matching the research goal.

Full accreditation of reference laboratories. Compliance with the WHO Laboratory Network standards and achieving performance indicators that make it possible to obtain full accreditation and ensure measles and rubella elimination verification.

Full accreditation of the national laboratory in accordance with WHO standards is the most important condition to provide reliable information which allows for the achieved measles and rubella elimination to be documented.

8. The meeting deems it necessary to remind that investigation of IgM antibodies is the basic instrument for verification of measles and rubella diagnosis. Considering the importance of laboratory data for assessment of the epidemiological situation, all the laboratories should conduct serological studies for measles and rubella and report the results to the surveillance system not later than four days after the specimens are delivered (for at least 80% of cases).

9. WHO accreditation shall guarantee the high quality of laboratory investigations. In exercising their tasks, the laboratories should seek compliance with all the required standards. Special consideration should be given to full implementation of the internal quality control system and timely reporting to WHO and the RRL.

10. For conducting the external quality control procedures, reference laboratories should submit specimens for re-testing to the RRL at least two times a year. Use of dry serum drop for re-testing has proved to be efficient in recent years and can be recommended to all countries with the aim to facilitate sending the specimen.

11. RRL and national laboratories which run molecular studies on measles and rubella should take part in the new programme on molecular study external quality assessment. WHO stands ready to provide the relevant primers and probes to support such studies.

12. The occurrence of measles outbreaks in vaccinated populations asks for usage of high-sensitivity lab tests to confirm diagnosis in vaccinated patients. There is a need to deepen the studies on measles re-infection and the role of vaccinated patients in spreading the infection; such studies should be conducted in coordination with WHO and the Measles/Rubella Global Laboratory.

13. In order to secure the high quality of studies at the national level, it is recommended that the national laboratories permanently exercise external control of the performance of the laboratories which participate in measles and rubella studies but which are not members of the WHO network. Information about other laboratories (except NRL and SNL), which conduct measles and rubella studies should be channeled into the surveillance system and be reflected in the relevant section of the annual report of the certification committee.

Molecular surveillance. Expanding molecular-genetic studies of measles and rubella viruses and providing data to MeaNS and RubeNS databases

Molecular and genetic data play a key role in the infection elimination verification process. In order to expand data on molecular epidemiology of measles and rubella it is necessary to enhance collection of specimens for virological studies and to ensure timely reporting of molecular data.

14. Molecular and genetic investigation of viruses is an integral component of the laboratory part of measles and rubella epidemiological surveillance. It is recommended to all the countries to enhance collection of specimens for virological studies on measles and rubella and select for further genotyping epidemiologically informative specimens only.

15. Laboratories should provide for genotyping of at least 80% of epidemiologically identified chains of virus transmission and immediately provide sequencing data to the national surveillance system and within two months of specimen reception, to the WHO MeaNS and RubeNS genotyping databases.

16. If the laboratories do not conduct genotyping, clinical specimens or PCR-products should be timely submitted to the RRL. It is recommended to use Fast Technology Analysis (FTA) cards for resending virological specimens. FTA cards do not require cold-chain and biosafety conditions to be observed and they do not incur high costs. The accompanying documents should state vaccination history of the patient and epidemiological update of the region.

17. With the purpose of improving timely and complete presentation of sequencing data to the MeaNS and RubeNS databases and to ease the workload of the RRL, national laboratories are encouraged to submit the nucleotide sequences of their viruses in the databases.

The WHO Regional Office for Europe

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