

Report on the

# Meeting on the measles and rubella network in the Eastern Mediterranean Region

Cairo, Egypt  
2 December 2007



**World Health  
Organization**

Regional Office for the Eastern Mediterranean

Report on the

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## 1. INTRODUCTION

The World Health Organization (WHO) Regional Office for the Eastern Mediterranean held a regional meeting on the measles and rubella network in Cairo, Egypt, on 2 December 2007. The objectives of the meeting were to:

- review progress made in laboratory surveillance on measles/rubella in the Region;
- provide updated technical information on laboratory issues related to measles elimination goals in the Region;
- discuss further strengthening and the way forward on the measles and rubella laboratory network in the Region.

The opening address of Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean, was delivered by Dr Ezzeddine Mohsni, Regional Adviser, Vaccine Preventable Diseases and Immunization (VPI). Dr Gezairy emphasized the importance of the laboratory in the elimination of measles and said that considerable progress had been made in establishing the laboratory network in the Region. One of the many developments this year had been the increased capacity-building of the laboratory network for virus detection and genotyping which had improved the mapping of endemic circulation measles virus in a number of countries where such information had not been previously available.

As more countries of the Region moved towards the measles elimination phase, the need for enhanced surveillance, allied to rapid and accurate measles virus diagnosis was required to monitor the success of this important programme. In this regard, it was vital that the plan of action for surveillance was coordinated between epidemiologists and laboratory personnel, and vigilance and readiness to detect any circulating measles virus were conducted in a timely manner.

The programme and list of participants are included as Annexes 1 and 2, respectively.

## 2. OVERVIEW OF MEASLES/RUBELLA LABORATORY NETWORK ACTIVITIES

### 2.1 Overview of global laboratory networks

*Mr David Featherstone, WHO headquarters*

The WHO measles and rubella laboratory network (LabNet) was established to provide a standardized testing and reporting structure and a global quality assurance programme. The LabNet now includes 688 IgM testing laboratories serving 164 countries, and the network continues to expand, although more slowly. More than 180 000 serum samples were tested for measles IgM globally in 2006, and most negative samples also tested for rubella. Of the 200 national and regional reference laboratories which participated in the global proficiency test, 98.5% achieved a score of  $\geq 90\%$ . The Pan-American Health Organization (PAHO) laboratories participated in the global proficiency testing programme for the first time in 2006.

Training workshops have been conducted for new and existing laboratories for the introduction of new techniques. For the first time training has been expanded to include

molecular techniques to improve molecular epidemiological surveillance. One such workshop was held at the Oman regional reference laboratory in April 2007 for key regional laboratories and has resulted in an additional three countries reporting measles virus genotype information in the six months following the workshop.

A measles and rubella genotype database has been established in WHO headquarters and has had measles viruses submitted from 59 countries from all six WHO Regions, though there are still some surveillance gaps. Fifteen of 23 measles genotypes are represented in the database but of the more than 2500 viruses submitted only 7% have been reported with a GenBank accession number, as recommended. The rubella virus database has only 128 viruses submitted to date reflecting both collecting samples from suspected rubella cases and difficulty in identifying the rubella virus compared with the measles virus. Virological surveillance, in parallel with standard epidemiological investigation data, can help document pathways of viral transmission and help determine the effectiveness of control programmes.

## **2.2 Overview of the regional laboratory network**

*Dr Hinda Ahmed WHO/EMRO*

The WHO Regional Office for the Eastern Mediterranean has established a regional goal to eliminate measles by 2010, and laboratory support of surveillance is an indispensable element of the programme. In the Region, the development of the laboratory network has been an evolving process over the past two years. To date, all national measles laboratory in countries of the Region have full serological capacity. Two regional reference laboratories, two subregional reference laboratories and 22 national measles laboratories have been designated for the diagnosis of measles and rubella.

For measles laboratory diagnosis it is recommended that measles be diagnosed using serological methods which measure virus-specific IgM antibody in serum samples. Most of the existing regional measles/rubella laboratory network functions well, as has been seen by the significant improvement in the quality of case-based surveillance and laboratory data. All laboratory networks, except one which participated in proficiency testing for measles and rubella serology, passed with a result of 95% or more. Quality assurance procedures are being implemented at national measles laboratories and the number of national measles laboratories sending samples for quality control is increasing, 19 laboratories participated in serum referral to regional reference laboratories for validation obtaining over 90% concordance. On-site visits and an accreditation review have been carried out over the past two years and 17 countries passed the accreditation review. Regional virus detection and genotyping has been strengthened. Recently, a workshop on laboratory diagnosis for measles and rubella virus detection and genotyping was organized by the Regional Office and was held in the regional reference laboratory in Muscat, Oman, which was attended by 10 participants from nine countries which have virus isolation or PCR facilities.

However, some countries in the Region are not fully utilizing the laboratories by not testing more than 80% of measles suspected cases recommended in the measles elimination phase. Five to ten blood samples are required to be collected of initial cases during outbreaks for laboratory confirmation and to collect specimens for virus isolation/detection and

genotyping. Strong communication and cooperation between laboratory professional and surveillance officers is the key element for the success of any surveillance programme.

Constraints and challenges are logistic problems for specimen referral from districts to national laboratories and to regional reference laboratories for quality control, sharing information in a more timely manner, the need for close collaboration of laboratory staff and epidemiologists, the collection of clinical samples during outbreaks for virus isolation in laboratories with sufficient capacity or to send clinical samples to the regional reference laboratory to facilitate genotyping of circulating virus in the country or the Region. There is a need to establish subnational laboratories where necessary to appropriately monitor and report disease from remote areas and difficult geographical areas of large countries in the Region. Also, more appropriately, alternative sampling, such as dried blood or dried serum, which has shown good sensitivity and specificity in several studies needs to be introduced in areas with logistical problems.

The Regional Office is committed to assisting in building mechanisms for strengthening laboratory network improvements, linking laboratory data and activities to surveillance, conducting periodic reviewing quality assurance through an accreditation and proficiency testing programme, validating results, providing supplies and equipment, feedback or laboratory reports and building the capacity of laboratory personnel.

### *Discussion*

The possibility of sending specimens using the WHO pouch system was brought to the attention of participants. The huge difference between the number of measles and rubella samples tested was questioned. The procedure is to test first for measles and test the negative samples for rubella. The outbreak of rubella in some countries of the Region, such as Egypt, might be responsible for this difference.

## **3. COUNTRY PRESENTATIONS**

### **3.1 Afghanistan**

*Dr Agha Gul Dost*

In 2007 the national measles laboratories tested 146 samples of which two were measles IgM positive and 20 samples gave equivocal results due to inadequate specimen condition, manual washing and using expired kits. Challenges facing the national measles laboratory are.

- low adequacy of specimens (42% of samples were inadequate);
- serum specimens taken from only 37% of patients;
- difficulty in transporting specimens from remote areas and the lack of regional laboratories;
- low knowledge of clinicians and laboratory staff;
- shortage of kits;
- delay in feedback because of non-availability of an Internet connection in most provinces.

The action plan to improve laboratory measles surveillance includes:

- conducting training for the health facility in charge and laboratory staff;
- conducting regular meetings and training workshops for clinicians and laboratory personnel together to overcome constraints;
- collecting blood specimens from all sporadic cases and 5–10 samples from outbreak areas;
- monitoring strictly all the indicators of laboratory performance, including quality control/quality assurance;
- sending throat swabs to the regional reference laboratory (Pakistan) to identify the measles virus genotype;
- ensuring regular maintenance of laboratory equipment;
- ensuring regular communication and feedback;
- increasing the awareness of the community and health staff to report suspected measles cases in silent districts.

### *Discussion*

A large number of equivocal results may have been obtained due to the use of expired reagents. The breakage of washing machines may also be another reason.

### **3.2 Sudan**

*Dr Rehab Abdel Aziz Alaib*

In Sudan, the laboratory involvement in measles surveillance has improved and the laboratory focal point is a member of the technical advisory group. The laboratory shares with the technical advisory group and the Expanded Programme on Immunization (EPI) surveillance data. In 2007, 566 serum samples were collected from suspected measles cases, of these 149 specimens were measles IgM positive and four were equivocal. The laboratory participates in specimen transfer for quality control and the accuracy of specimen validation results is over 90%. Some of the challenges include: lack of professional maintenance and repair of equipment; the need to ensure sustainable financial support; staff upgrading (particularly PCR); and lack of a data management system.

### **3.3 Jordan**

*Ms Samar Jamal Sadeddin*

Laboratory activity is up to standard and has been accredited. The laboratory has tested 245 suspected cases of measles, of which 31 were measles IgM positive with four equivocals. In 2007, a measles virus isolate was sent for genotyping and was found to be genotype D4. The measles laboratory is not linked to the surveillance database and does not receive complete data elements of the cases. Some of the challenges include that the measles laboratory focal point is not a member of the measles technical advisory group, there is a lack of clear communication with surveillance unit, the laboratory is not represented officially in the technical advisory group, and there is a lack of sufficiently-trained laboratory staff in the PCR unit.

### **3.4 Morocco**

*Dr Amal Alla*

The serology capacity and activities of the measles and rubella national laboratory began in 2002 and until now 645 serums have been analysed. Virus isolation started in 1999, and 24 strains were isolated from 1999 to 2007. Analysis of 33 measles strains collected from 1998 to 2005 with the collaboration of the Centers for Disease Control (CDC), Atlanta, and the Inserm Institute in Lyon identified measles genotype C2 as endemic strain genotype (B3.2, D7 and D8) and imported.

As a result of the poor notification of measles cases in the surveillance system, nine samples were sent and analysed by the national measles laboratory in 2006. To tackle this problem sentinel surveillance sites were established in five provinces in different geographical areas and awareness-raising meetings were conducted with the collaboration of the epidemiological department in sentinel sites. This has resulted in improvements in the measles surveillance system, and as of 2007, the laboratory has received 192 serum samples of which 64 were measles IgM positive and 10 measles virus were isolated. Challenges include the: difficulty in collecting blood specimens from young children (oral fluid sampling should be introduced); the need for a specific measles programme for data management of the laboratory network; and difficulties of specimen shipment (Fedex and DHL refuse the shipment).

## **4. LABORATORY QUALITY ISSUES**

### **4.1 Laboratory procedures and quality insurance**

*Dr Hinda Ahmed, WHO/EMRO*

The available information on laboratories is very encouraging in terms of the accuracy of test validation and the achievement of high proficiency. However, some managerial issues need to be given attention in order to fulfil the six requirements for laboratory accreditation, including:

- Test results are reported by the laboratory on at least 80% of measles IgM samples within 7 days of receipt.
- Serological tests are performed on at least 50 serological specimens annually.
- The accuracy of measles and rubella IgM detection is at least 90%.
- Internal quality control procedures for IgM assays are implemented.
- The score on the most recent WHO approved proficiency test is at least 90%.
- The score from the annual on-site review of laboratory operating procedures and practices is at least 80%.

The laboratory has a critical role to play in the surveillance of measles and rubella. The coordination of EPI staff and laboratory staff is very important. Standardization of laboratory activities is key to a successful network and it is essential that all laboratories are testing accurately, reporting in a timely manner and meeting performance criteria.

## **4.2 Challenge of shipment of samples into the network**

*Dr Suleiman Al-Busaidy*

When looking into the challenges facing shipments of samples and infectious materials into the network, many factors need to be considered, such as regulations, other national and international bodies, international transportation and country clearance. Within a country, local shipping of samples may be hampered through delays in shipment and inadequate refrigeration, which in turn, affects sample quality adversely. When looking into intercountry sample shipment for confirmation and isolation, courier services and airline transportation methods are required. The reliance on these methods of shipment has made the transportation industry increasingly reluctant to accept such materials, citing safety concerns. This concern is due to the possibility of their becoming infected as a result of exposure to infectious microorganisms that may escape from broken, leaking or improper packaging.

Concern has built up as a result of the media and the emergence of new infectious diseases. Although there are no recorded cases of illness attributed to the release of infectious substance or diagnostic specimens during transport, there are reported incidents of damage and shipment of unmarked or unidentified improperly-packed material which increases the overall potential of exposure. Nevertheless, damage to packaging may result in delays or the sample may not arrive at its destination for analysis.

Other factors that are to be considered in regard to the challenges of shipping samples include the availability of resources for shipment, funds to support these shipments, training on the guidelines for safe transportation, and awareness of the transportation regulations. Communication within a country or between countries, or even with regulatory transportation bodies, is another factor that needs to be considered. The main factor for shipment, however, is awareness of biosafety regulations and international shipment regulations.

Once regulations are applied and are followed appropriately, and the definition, classification and identification of infectious substances are clarified, this will reduce the challenges being faced by sample shipments. Nonetheless, being aware of packaging regulations and the adequate labelling of documentation will contribute to reducing the problems of shipment. Additionally, the alternative sampling method is another future approach which may help in the shipment of samples.

## **5. PROGRESS OF IMPROVED MEASLES VIROLOGICAL SURVEILLANCE IN THE REGION**

### **5.1 Measles molecular epidemiology and the role of virus isolation and rubella genotype**

*Dr Hind Triki*

In addition to serological confirmation of suspected measles cases, measles laboratories are requested to provide data on the genetic characteristics of circulating viruses. These data help to document persistent or interruption of circulation of endemic viruses and to identify the origin of imported viruses. They are obtained through virus isolation and sequencing virus RNA. At the beginning of 2000, circulating genotypes in most countries of the Region were not identified. Substantial progress has been made in the previous five years. A total of 100 virus isolates or PCR products could be assessed for genotype identification in the two regional laboratories, 66 in the regional reference laboratory in Tunis and 34 in the regional reference laboratory (Oman). The samples originated from 10 countries: Egypt, Islamic Republic of Iran, Iraq, Jordan, Kuwait, Libyan Arab Jamahiriya, Oman, Qatar, Sudan and Syrian Arab Republic. Molecular investigations concluded genotype D4 in most countries: Egypt, Islamic Republic of Iran, Iraq, Jordan, Qatar and Syrian Arab Republic. Genotype B3 was detected during the measles outbreaks in the Libyan Arab Jamahiriya and Sudan in 2007. Genotype D5 was identified in one measles sporadic case in Oman 2007. Several virus isolates during different years are available from Iraq and Islamic Republic of Iran which allows analysis of the trends and genetic evolution of circulating strains. Despite this progress, greater efforts are needed. Virus isolates are available from only 10 of the 22 countries in the Region and, for most countries, only one or very few isolates are available. Insufficient data are available on the genotypes of circulating rubella viruses in most countries of the Region.

### **5.2 Importance of continuation of measles virological surveillance**

*Dr T. Mukhtari*

In the Islamic Republic of Iran the goal of measles elimination has been strengthened, more than 80% of the measles suspected cases were tested in 2007; 758 serum samples were tested and only four were measles IgM positive. There is good coordination and collaboration between the EPI, surveillance and the laboratory. The constraints are: retiring professional staff; inadequate staff; purchase of materials from foreign countries is problematic; lack of specific laboratory budget line; and shipping isolates.

### **5.3 Alternative diagnostic and sampling methods**

*Mr David Featherstone WHO headquarters*

The use of dried blood and oral fluid samples, as alternatives to serum, has recently been evaluated for rubella to build on the considerable data available for measles. These sampling techniques may have a role in countries that have challenges in collecting venepuncture blood from infants and/or transporting samples under reverse cold chain to the testing laboratory. Good concordance of both oral fluid and dried blood samples with parallel serum samples has been found for measles and rubella using commercially available assays.

IgM in dried blood and oral fluid is relatively stable at 37 °C and 42 °C for 3–4 days, although some decline in absorbance values is detected at higher temperatures over 2 weeks. Oral fluid samples can increase the sensitivity of molecular surveillance for measles and rubella viruses, with evidence from the United Kingdom and the Netherlands that it is possible to obtain sequence information from cases up to 30 days after rash onset. Serum is still considered the gold standard for IgM detection though alternative sampling techniques can enhance surveillance where it is difficult to collect and/or transport serum and oral fluid samples can be especially useful for molecular surveillance.

There is good evidence that using the dried blood technique for serum samples could greatly assist laboratories shipping confirmatory samples to their reference laboratories for quality assurance purposes. The global specialized laboratories at CDC, Atlanta, and the Health Protection Agency, London, have looked at a limited number of dried serum samples using two different techniques. Concordance between the IgM detection of liquid serum and dried serum was very high, though HPA found absorbance values showed a slight drop at 37 °C after 3 weeks at approximately 2% per day. Further validation is warranted under "field" conditions.

## **6. RECOMMENDATIONS**

1. Provide training opportunities to countries where staff turnover is a problem and to countries with adequate facilities to keep strengthening the capacity of measles and rubella virus detection.
2. Use Vero/SLAM cells for measles and rubella virus isolation in order to avoid the biohazard of the B95a cell line.
3. Enhance the collection of clinical specimens for measles and rubella virus detection and genotyping.

### *To sequencing laboratories*

4. Submit viruses sequence information to the WHO genotype database on a timely basis.
5. Collect more field data to evaluate dry serum samples. WHO should provide protocol for the sampling and testing.
6. Submit epidemiologic and laboratory data using the shared data transfer Excel sheet for specimen referral for validation or genotyping.
7. Provide data management training in countries in need of support to streamline measles surveillance data.
8. Conduct annual measles and rubella laboratory network meetings.

**Annex 1****PROGRAMME****Sunday, 2 December 2007**

08:00–08:45 Registration

08:45–09:00 Opening session

- Opening remarks
- Introduction and election of Officers
- Adoption of the agenda

**Session 1. Overview of measles/rubella laboratory network activities**

09:00–09:10 Global laboratories network

*Mr D. Featherstone  
WHO/HQ*

09:10–09:20 Regional laboratory network

*Dr H. Ahmed  
WHO/EMRO*09:20–09:40

- Afghanistan
- Sudan

*Mr Faisal Afghan  
Mrs R. Alaib*09:40–10:00

- Jordan
- Morocco

*Ms S. Saadeldin  
Dr A. Alla*10:00–10:20

- Kuwait
- Saudi Arabia

*Dr S. Al Mufti  
Mr M. Al-Amri*

10:20–10:40 Discussion

**Annex 2**

**LIST OF PARTICIPANTS**

**AFGHANISTAN**

Dr Agha Gul Dost  
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**Riyadh**

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Ministry of Health Puntland Laboratory

**Puntland**

**SUDAN**

Dr Rehab Abdel Aziz Alaib

Medical Laboratory Technologist NHL

Federal Ministry of Health

**Khartoum**

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**Damascus**

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Reference laboratory for Measles and Rubella Diagnosis

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