Expansion of the Global Measles and Rubella Laboratory Network 2005–09

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Enhancing measles surveillance with integration of epidemiologic and laboratory information is one of the key strategies for accelerated measles control and elimination. The World Health Organization (WHO) Global Measles and Rubella Laboratory Network (LabNet) has been developed since 2000 to currently include 690 laboratories serving 183 countries. The LabNet testing strategy follows well-validated, standardized procedures for confirming suspected cases and for monitoring measles and rubella virus transmission patterns. The strength of the LabNet is a strong quality assurance program that monitors the performance of all laboratories through annual proficiency testing and continuous assessment. In the 5-year period 2005–2009, the results of >1 million measles immunoglobulin M (IgM) tests have been reported by the LabNet and, in addition, sequence information on >7000 measles and 600 rubella viruses has been shared. Progress with the development of the LabNet during 2005–2009 is discussed.

Progress with measles control since the year 2000 has been dramatic. It is estimated that >4.5 million measles deaths have been prevented as of 2008 through implementation of the strategies developed by WHO and the United Nations Children’s Fund [1]. Four World Health Organization (WHO) regions (Americas [AMR], Eastern Mediterranean [EMR], European [EUR] and Western Pacific [WPR]) have established elimination goals, and the WHO African Region (AFR) has developed strategies leading to an elimination goal that they term “pre-elimination.” The Southeast Asian Region (SEAR) has a mortality reduction goal.

Enhancing measles surveillance with the integration of epidemiologic and laboratory information is one of the key strategies for accelerated measles control and elimination, and the WHO Measles and Rubella Laboratory Network (LabNet) has been established to provide timely and accurate data for the surveillance of measles and rubella.

FUNCTION OF THE GLOBAL MEASLES AND RUBELLA LABORATORY NETWORK

The LabNet plays a critical role in confirming suspected cases of measles through the detection of specific immunoglobulin M (IgM) and testing measles-negative cases for evidence of rubella infection. In addition, identification and characterization of measles and
rubella viruses provide information about the origin of outbreaks, whether endemic or imported, which is required for monitoring progress toward elimination.

As disease prevalence falls, medical personnel become less experienced in recognizing clinical measles and rubella, thus increasing the importance of laboratory methods to distinguish measles and rubella from other diseases with similar clinical presentations. The strategy of testing measles-negative cases for rubella was developed primarily to enhance the measles surveillance system in the WHO Region of the Americas. This strategy revealed previously unrecognized widespread circulation of rubella virus in many countries in the region. Furthermore, various studies indicated that the disease burden of congenital rubella syndrome was significant in the Americas and underscored significant morbidity from rubella and congenital rubella syndrome in the Western hemisphere [2–4]. After measles elimination was accomplished in the AMR in 2002, a rubella elimination program was established using the same strategies, including laboratory-based surveillance.

As of July 2010, the LabNet consists of 690 laboratories, all of which follow a standardized set of testing protocols and reporting procedures with a strong focus on quality assurance. The structure of the LabNet was based on the successful polio laboratory network [5] and was established in 4 tiers of laboratories: national level (n = 162), regional reference (n = 19), global specialized (n = 3), and subnational level (n = 506) (Figure 1).

The cornerstones of the LabNet are the national-level laboratories, which provide timely and accurate case confirmation of suspected measles and/or rubella cases using standard enzyme-linked immunosorbent assay (ELISA)-based IgM detection from serum samples collected at first contact with the health system. These laboratories are closely linked with the national disease control programs and often are responsible for surveillance of other exanthematous diseases as well, including dengue, parvovirus B19, and human herpesvirus type 6. Differential testing for other exanthematous disease can improve the interpretation of measles and rubella test results, especially in low-incidence settings. By June 2010, 160 of the 193 member states of WHO had established national laboratories with a further 24, mostly countries with small populations, served by another country’s laboratory. Some large countries or those with logistical challenges in transporting samples have established laboratories at the subnational level to facilitate the timely testing and reporting of results. For example, China has established 362 laboratories at the first (n=31) and second (n=331) administrative levels (provincial and prefecture, respectively) [6]. In 2009, the Chinese laboratory network tested more than 86,000 serum samples for measles and rubella IgM antibodies following the same testing strategies and comprehensive quality assurance and accreditation assessments as the remainder of the global LabNet.

Two or three regional reference laboratories have been selected in each WHO region. The regional reference laboratories are regional centers of excellence that support the capacity building of the national-level laboratories under their
responsibility and provide confirmatory testing of a proportion of the samples tested by the national-level laboratories. They also support genetic characterization of circulating wild-type viruses to help monitor viral transmission pathways and progress toward measles elimination. Global specialized laboratories are global centers of excellence that have been established in Japan, the United Kingdom, and the United States. The global specialized laboratories contribute to the standardization of procedures and protocols, development and validation of new techniques, and global capacity building through focused training, and some provide financial support.

LABORATORY TESTING STRATEGIES

The standard LabNet test for measles and rubella confirmation is the detection of IgM in serum, oral fluid, or eluates of dried blood spots collected from suspected cases at their first contact with a health facility [7]. For molecular testing, preferred samples are throat swabs or oral fluid for direct virus detection of viral RNA by reverse-transcription polymerase chain reaction assay. Throat swab samples are also used to isolate measles virus in cell culture. Urine samples and peripheral blood mononuclear cells can also be used for virus culture, although in practice these have proven to be more difficult to collect, to transport, and to process successfully. The LabNet has introduced the Vero/hSLAM cell line as the standard for virus isolation, provided by Dr Yanagi [8]. This cell line was demonstrated to be sensitive for both wild-type measles and rubella virus culture [9](unpublished data from LabNet) and has proven to be easily handled in the LabNet.

ELISA-based IgM detection in serum was established as an accurate, simple, and rapid procedure to confirm cases of suspected measles or rubella. Various commercial assays were comprehensively validated [10], and assays with high sensitivity and specificity are currently used by most national laboratories in the LabNet. National and subnational laboratories in some countries use locally produced commercial assays. Locally produced assays in the United States, the Netherlands, Japan [11, 12], China, and the Russian Federation have been evaluated (unpublished data from LabNet), some of which have shown sensitivity and specificity comparable with the commercial assays used by most laboratories in the LabNet.

A serum sample collected at first contact with a health facility is considered the gold standard for measles IgM ELISA detection, because sensitivity has been reported to be 77% within 72 hours after rash onset and 100% at 4 days [13]. Oral fluid and dried blood samples have been used for enhancing the diagnosis of numerous diseases [14–20] and have been considered for use in the LabNet. The LabNet has comprehensively evaluated oral fluid and dried blood sampling techniques, using IgM detection for measles and rubella diagnosis and viral RNA detection and sequencing for molecular surveillance, determining their temperature stability outside the cold chain, and assessing their ease of use [21, 22]. In 2010, five African countries (Benin, Cote d’Ivoire, Kenya, Malawi, and Zimbabwe) began evaluating oral fluid sampling techniques as a mechanism to enhance surveillance through use of a single sample for both case confirmation (IgM) and molecular surveillance (RNA). If results from the trial are encouraging, this technique will be considered for introduction to a larger number of countries in the region. Several countries within the WHO Southeast Asian Region are also considering trying the use of oral fluid samples to enhance surveillance.

QUALITY ASSURANCE AND SURVEILLANCE INDICATORS

Quality assurance is a major strength of the LabNet and includes confirmatory testing of national and subnational laboratory results, a global proficiency testing program, implementation of in-house quality control measures, and an accreditation program. The global proficiency testing program for the LabNet began in 2000, and initially 38 laboratories were involved [23]. The proficiency testing serum panels consist of 20 samples from patients with recent measles, rubella, or other exanthema and are produced and analyzed by the Victorian Infectious Diseases Reference Laboratory in Melbourne, Australia, one of the LabNet’s regional reference laboratories for the Western Pacific Region. In 2009, there were 220 laboratories participating in the proficiency testing program, including all national, regional reference, and global specialized laboratories, the 31 Chinese provincial laboratories, and all subnational laboratories in the Russian Federation, Turkey, and Ukraine. For measles, 98% of laboratories achieved a passing score (≥90%), with 84% of laboratories achieving 100%. For rubella, the scores were 99% and 94%, respectively.

Because the proficiency testing program provides a snapshot of the accuracy of IgM testing, a continual assessment of a laboratory’s testing accuracy was established through a confirmatory testing program. This program requires national and subnational laboratories to forward annually a representative sample (approximately 10% of all samples) to the designated regional reference laboratory for repeat testing with an expected concordance of ≥90%. The WHO accreditation program monitors performance of laboratories on a continual basis, but the assessment is expected to be completed annually. Seven performance criteria are assessed: reporting timeliness of case confirmation results, reporting timeliness of genetic characterization data, accuracy of results compared with the reference laboratory, implementation of specified quality control procedures, a passing score in the annual proficiency test, a minimum number of 50 tests performed per year, and a passing score on a comprehensive onsite review of the laboratory’s work practices. National laboratories and reference laboratories have similar...
criteria, but the expected passing scores are higher for the reference laboratories.

The function of the accreditation program is not only to monitor the quality of the LabNet but also, and most importantly, to identify any capacity building that is needed and to troubleshoot any problems that may have arisen. A laboratory that achieves less than the passing score on any one of the performance criteria will work with the WHO regional laboratory coordinator to identify areas in which improvement is needed and to develop and implement a workplan to reach the full accreditation benchmarks as soon as possible. Quality assurance of serologic testing is comprehensive, and plans have been developed for a similar program to ensure the accuracy of molecular techniques and the sensitivity of virus culture.

A number of the surveillance indicators that are used to assess the quality of measles surveillance are dependant on laboratory function. These indicators include the percentage of cases with an adequate sample, the percentage of samples that arrive in the laboratory within 3 days, the percentage of results reported within 7 days, and the percentage of suspected cases discarded by the laboratory. LabNet member laboratories have achieved the target of 80% for most of these indicators; however, in some regions obtaining adequate samples is difficult because of logistic problems or inadequate case investigations, and often timely transportation of samples to the laboratory is difficult to achieve.

**LABNET COORDINATION**

Coordination of the LabNet is a key facet to a successfully functioning network. Communication among all levels of laboratories is carried out through regular meetings, workshops, and information exchange through telephone and email. Global LabNet meetings have been held annually since 2001, and regional laboratory coordination meetings are held in each of the 6 WHO Regions every 1–3 years. Each of the 6 WHO Regions has a dedicated laboratory coordinator with expertise in virology and experience with managing laboratory networks. These coordinators are positioned in the regional or subregional offices, and one is located in WHO headquarters. Their primary roles are to work with Ministries of Health to assess and to select appropriate laboratories, to expand the capacity and capability of LabNet through standardized training activities, to ensure laboratories are provided with appropriate equipment, to provide standardized supplies, to establish regular reporting structures, to perform accreditation reviews, and to coordinate the resolution of any performance issues. The regional reference and global specialized laboratories play a key role in strengthening capacity through support of training, providing troubleshooting advice, and developing and validating new technologies to improve laboratory-based surveillance.

**LABNET INTEGRATION AND CAPACITY BUILDING**

The LabNet was built on the experience gained from establishing the polio laboratory network and capitalized on the same infrastructure in a number of countries. In turn, the measles and rubella LabNet has been used to build similar laboratory-based surveillance networks globally. As of June 2010, these include laboratories focusing on yellow fever in West and Central Africa (n=23), Japanese encephalitis in SEAR (n=13) and WPR (n=9) (Figure 1), and human papillomavirus infection (n=10) globally. The rotavirus laboratory network was established in the late 1990s in WPR and SEAR and extended to all WHO regions by 2007, using a similar proficiency testing and data sharing model [24–26]. The pediatric bacterial meningitis laboratory network for *Haemophilus influenzae* type b surveillance, dating from the early 2000s in the African region, used a similar proficiency testing and data sharing program. There is considerable overlap in personnel, laboratories, and institutions used for these different surveillance activities. A number of measles LabNet personnel have also been involved in the enhanced global surveillance for pandemic influenza and in establishing routine surveillance for seasonal influenza. The more recently initiated global disease surveillance programs benefit from the capacity developed in establishing the polio and measles and rubella LabNets, especially through the linkages developed with WHO and specialized laboratories, the provision of standardized equipment and supplies, and the skills developed through the extensive training programs implemented.

The capacity of the Measles and Rubella LabNet has developed rapidly, especially in the past 5 years. The number of samples tested for measles IgM has increased from 119,000 in 2005 to 173,000 in 2009, with most of the increase in the Western Pacific Region. The number of samples from SEAR is expected to increase considerably after India begins case-based surveillance (Figure 2). Approximately 67% of all samples were also tested for rubella IgM, with approximately 400,000 IgM tests being performed in total by the LabNet in 2008. In 2009, there were 193,120 samples received for testing in the LabNet, of which 327,082 tests were performed for measles and/or rubella IgM antibodies (Table 1).

For monitoring and tracking molecular surveillance data, genotype databases for measles and rubella were established at WHO headquarters in 2006, which include genotype and basic epidemiologic data. As of 1 June 2010, 7675 measles viruses from 123 countries have been sequenced and key variables submitted to this database, and details on 600 rubella viruses from 36 countries have been submitted [27, 28]. In 2008, a joint Health Protection Agency and WHO measles sequence database (MeaNS) was also established, which included sequences from 4,200 viruses by 1 June 2010.
Resources for the LabNet are provided largely through bilateral support mechanisms. Countries provide the infrastructure costs and cover staff salaries and overheads; and WHO, with the aid of its partners—a key one of which is the US Centers for Disease Control and Prevention—supports training activities and assists laboratories in obtaining optimal levels of equipment and supplies [29]. In some WHO Regions, operational costs may be provided to priority countries in addition to supplies and training.

Discussion

The development of LabNet has progressed rapidly during the past 10 years. The number of laboratories has increased from 60 in 2000 to 690 in 2010 but has likely reached a plateau, because almost all countries have established laboratory-based measles surveillance. However, the complexity of testing will continue to increase as molecular surveillance to determine transmission patterns and to distinguish between imported and endemic measles cases is fully embraced. LabNet testing is critical for adding specificity to surveillance information when incidence

Table 1. Reported Laboratory Workload and Performance of Laboratories Participating in the LabNet by World Health Organization (WHO) Region, 2009

<table>
<thead>
<tr>
<th>WHO Region</th>
<th>No. of serum samples received</th>
<th>No. of samples tested</th>
<th>No. of samples IgM positive</th>
<th>% Positive</th>
<th>No. of samples tested</th>
<th>No. of samples IgM positive</th>
<th>% Positive</th>
<th>Results reported within 7 days, a %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFR</td>
<td>36,558</td>
<td>35,242</td>
<td>10,088</td>
<td>29</td>
<td>29,611</td>
<td>5157</td>
<td>17</td>
<td>59</td>
</tr>
<tr>
<td>AMR</td>
<td>20,668</td>
<td>20,415</td>
<td>89</td>
<td>0.4</td>
<td>20,415</td>
<td>18</td>
<td>0.09</td>
<td>84</td>
</tr>
<tr>
<td>EMR</td>
<td>13,222</td>
<td>13,222</td>
<td>5950</td>
<td>45</td>
<td>11,824</td>
<td>770</td>
<td>7</td>
<td>95</td>
</tr>
<tr>
<td>EUR</td>
<td>24,929</td>
<td>18,665</td>
<td>3314</td>
<td>18</td>
<td>17,660</td>
<td>1094</td>
<td>6</td>
<td>78</td>
</tr>
<tr>
<td>SEAR</td>
<td>6561</td>
<td>6128</td>
<td>1103</td>
<td>18</td>
<td>5368</td>
<td>2656</td>
<td>49</td>
<td>70</td>
</tr>
<tr>
<td>WPR</td>
<td>91,182</td>
<td>71,426</td>
<td>34,412</td>
<td>48</td>
<td>49,084</td>
<td>12,787</td>
<td>26</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Total</td>
<td>193,120</td>
<td>165,098</td>
<td>54,956</td>
<td>33</td>
<td>133,962</td>
<td>22,482</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. AFR, African Region; AMR, Americas Region; EMR, Eastern Mediterranean Region; EUR, European Region; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

* For the AMR, percentage of results reported within 4 days.
decreases and the predictive value of clinical diagnosis diminishes. In addition, monitoring progress toward elimination through sequence analysis of currently circulating wild-type viruses is needed to determine whether a virus is endemic or imported.

Despite the large improvement in molecular surveillance, gaps in baseline molecular surveillance for measles still exist and no genotype data are available for 72 countries, although 25 of these are from the Region of the Americas, which has already eliminated measles, and 20 are small countries with a very low incidence of measles. Gathering information on baseline rubella virus sequence data is still at an early stage of development, with no data reported from 158 countries.

As countries progress toward elimination of measles, laboratories will be required to provide genotype information from 80% of the chains of transmission. Achieving this target will require close coordination between those conducting case investigations and the LabNet and will increase the number of samples that must be processed and tested by the laboratory. Also, in elimination or pre-elimination settings, rapid confirmation of rashes due to vaccine reactions using molecular techniques will become increasingly important.

The positive predictive value of the procedure of using a single IgM ELISA on serum for confirming suspected measles and rubella cases in the LabNet is high when incidence of disease is high, but concerns have been raised as to whether these testing procedures are appropriate under low-incidence settings, because the positive predictive value decreases significantly [30]. Although the WHO Region of the Americas has successfully documented elimination of both measles and rubella using these testing strategies, the LabNet is investigating procedures that may improve the positive predictive value in low-incidence settings. In addition, specialized serologic testing, such as IgG avidity assays and plaque-reduction neutralization assays, are used in more advanced laboratory settings to aid in the classification of more difficult cases that sometimes arise in low-incidence settings [31, 32].

CHALLENGES

As of 2009, only 12 (Algeria, Comoros, Guinea Bissau, India, Mauritius, Monaco, Morocco, Pakistan, San Marino, Sao Tome and Principe, Seychelles, and Somalia.) of the 193 member states of WHO have yet to implement case-based surveillance with laboratory confirmation; however, 2 are large-population countries: India and Pakistan. However, LabNet laboratories are performing measles outbreak confirmation in all 12 except Mauritius, San Marino, Sao Tome and Principe, and Seychelles. The LabNet has shown that it has the capacity to conduct up to 400,000 IgM tests annually (2008) for measles and rubella, and individual laboratories have responded to an almost 30-fold increase in weekly baseline testing rates. When all countries establish case-based surveillance and meet the minimum surveillance and testing criteria, the annual number of LabNet tests will increase by an estimated 70,000. The LabNet has the capacity to meet this increased throughput; however, finding the resources to procure additional kits and to train and to equip additional laboratories in the countries that have yet to meet the surveillance indicators will require increased advocacy efforts and more country commitment to supporting measles and rubella laboratory-based surveillance.

Despite progress in measles control, large measles outbreaks occurred in 2009 and 2010 in Bulgaria and several African countries. Testing all suspected cases during large outbreaks is not essential if countries follow WHO’s recommended guidelines to epidemiologically link suspected cases to laboratory-confirmed cases [33]. However, some countries continue to collect samples from almost all suspected cases during an outbreak, often increasing the laboratory’s workload by many-fold. For example, in 2009 South Africa experienced a large outbreak of measles that saw the national laboratory reporting almost 17,000 positive cases during a 12-month period starting in mid-2009 [34]. The laboratory tested approximately 50 serum samples per week before the outbreak but progressed to testing >1400 specimens per week at the peak of the outbreak, within a period of several months. Although the national laboratory performed exceptionally well in managing the heavy workload, it was put under considerable pressure to provide an acceptable turn-around time while at the same time maintaining high-quality results. Laboratories in other countries, including Botswana, Cambodia, Lesotho, Malawi, Philippines, Swaziland, Ukraine, Viet Nam, and Zimbabwe, have experienced a large increase in workload following outbreaks. Although the weekly increase in number of samples tested was less than in South Africa, the impact on increasing the reporting turnaroun time has been similar. Procuring sufficient test kits, maintaining quality, and timely reporting of results under these circumstances make a considerable challenge.

Some countries have a considerable number of private laboratories in the health sector that are used for testing suspected measles cases. Many of these laboratories are funded through health insurance and may test only for the specific diseases covered by the reimbursement. It has been reported from the European and Western Pacific regions that the large number of these laboratories in some countries makes it difficult to monitor the quality and accuracy of test procedures and to ensure regular reporting through the public health system. In the Region of the Americas, the United States reported that approximately 25,000 measles IgM tests were performed by private laboratories in 1997 [35]. External monitoring of quality control in commercial laboratories is a challenge, although in some cases, LabNet laboratories provide confirmatory and follow-up testing procedures.
Table 2. Laboratory-Based Training Workshops Held January 2009–June 2010 for Measles, Rubella, Yellow Fever, and Japanese Encephalitis Surveillance

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of countries</th>
<th>No. of laboratories participating</th>
<th>No. of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFR</td>
<td>24</td>
<td>26</td>
<td>38</td>
</tr>
<tr>
<td>EMR</td>
<td>11</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>SEAR</td>
<td>11</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>WPR</td>
<td>14</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>102</td>
<td>178</td>
</tr>
</tbody>
</table>

NOTE. AFR, African Region; EMR, Eastern Mediterranean Region; SEAR, Southeast Asian Region; WPR, Western Pacific Region.

As part of the quality assurance program, national and subnational laboratories are required to send a proportion of their IgM samples to their designated reference laboratory for confirmatory testing. The international shipment for these procedures has amounted to a considerable cost burden for some countries and has posed multiple logistic difficulties when samples are transported across national borders. The LabNet has validated the use of drying serum onto filter paper for temperature-stable shipment, thus reducing the cost of shipping under refrigeration temperature. Furthermore, dried serum samples are approved as exempt from dangerous goods requirements and regulations [36].

The LabNet has strengthened the health system by building capacity, strengthening laboratory infrastructure, and developing human resources. In the 18 months from 2009 to June 2010, >170 national and reference laboratory staff were trained to perform IgM ELISA procedures and polymerase chain reaction and sequencing techniques, and to improve data and laboratory management for measles, yellow fever, and Japanese encephalitis surveillance (Table 2). However, as countries strive to reach their measles elimination goals, partnership support for the LabNet must increase as the complexity of testing and number of samples increase.

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