Enhanced Laboratory Surveillance for the Elimination of Rubella and Congenital Rubella Syndrome in the Americas

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One of the reasons the 1997 Technical Advisory Group on Vaccine-Preventable Diseases recommended acceleration of rubella and congenital rubella syndrome (CRS) prevention efforts was the fact that the enhanced measles surveillance system in the Americas found that 25% of reported measles cases were laboratory-confirmed rubella cases. Until 1997, the laboratory network primarily focused on measles diagnosis. Since 1999, due to the accelerated rubella control and CRS prevention strategy, laboratories have supported the regional measles, rubella, and CRS elimination goals. The measles-rubella laboratory network established in the Americas provides timely confirmation or rejection of suspected measles and rubella cases, and determination of the genotypic characteristics of circulating virus strains, critical information for the programs. A quality assurance process has ensured high-quality performance of procedures in the network. Challenges are occurring, but the measles-rubella laboratory network continues to adapt as the requirements of the program change, demonstrating the high quality of the laboratories in support of public health activities and elimination goals.

BACKGROUND

In 1994, the region of the Americas adopted the goal of interrupting indigenous measles transmission in the Western hemisphere by the year 2000 [1]. To achieve this goal, the Pan American Health Organization (PAHO) developed elimination strategies that coupled measles vaccination with integrated field and laboratory surveillance [2]. The number of measles cases in the Americas declined more than 99%, from approximately 250,000 in 1990 to 165 confirmed cases reported in 3 countries in 2007 [3]. The last documented widespread endemic transmission of measles virus in the Americas was in Venezuela in 2002 [4]. Laboratory activities played an important role in measles elimination, as case confirmation and timely isolation of measles viruses, coupled with determinations of the genotypic characteristics of circulating strains, were critical information for supporting elimination.

Lessons learned from the polio laboratory network were applied in the development of the measles network, including the use of standardized laboratory testing and reagents, reporting procedures, and quality assurance processes. However, measles laboratories in the Americas were unable to fully exploit the capital invested and infrastructure developed for the polio laboratory network, largely because when the measles laboratory network was established in 1995, many countries already had established laboratories with well-trained personnel in measles diagnosis. In addition, the specimens and laboratory methods were quite different between polio and measles diagnostics.

In contrast, the expansion from measles to include rubella was much more feasible because methodological and operational procedures were similar. Indeed, strategies to eliminate rubella and congenital rubella...
syndrome (CRS) were built upon and integrated into the existing program toward measles elimination [5].

The purpose of the present paper is to describe the contribution of laboratory surveillance in support of rubella and CRS elimination in the Americas.

RUBELLA DIAGNOSIS IN THE CONTEXT OF MEASLES LABORATORY NETWORK

Since 1996, PAHO has recommended that all suspected measles cases that were measles immunoglobulin M (IgM) negative should be tested for rubella IgM antibodies in order to enhance the measles surveillance system. This strategy revealed previously unrecognized, widespread circulation of rubella virus (RV) in many countries in the Americas and indicated that the disease burden of CRS was significant. In response to this information on rubella and CRS, in 1997 the Technical Advisory Group on Vaccine-Preventable Diseases (TAG) recommended acceleration of rubella and CRS prevention efforts [6]. In 1999, the surveillance system developed for measles was officially expanded to a measles-rubella integrated surveillance system. Patients suspected of having measles or rubella infection had their sera specimens analyzed simultaneously for IgM for both measles virus or RV, or, alternatively, measles IgM negative specimens were tested for rubella IgM [7]. Because of the similarities of serologic diagnostic assay procedures for measles and rubella, the implementation of rubella serologic diagnosis in measles laboratories was not difficult.

However, procedures for isolation of wild-type RVs are quite different from those for wild-type measles viruses. The implementation of virus isolation and genetic characterization of wild-type RVs in the Americas has been more difficult than the implementation of serologic testing (see below).

DESCRIPTION OF THE LABORATORY NETWORK IN SUPPORT OF RUBELLA/CRS ELIMINATION

The PAHO measles-rubella laboratory network has 21 national laboratories, 114 subnational laboratories, 2 regional reference centers, and 1 global specialized laboratory (Figure 1). Eight countries in the region (Argentina, Bolivia, Brazil, Colombia, Ecuador, Mexico, Paraguay, and Venezuela), because of their large population sizes and geographic challenges, established networks of subnational laboratories working with oversight by the respective national laboratory. The national laboratory is responsible for the quality control of the subnational laboratories [8, 9]. For smaller countries, generally just the national laboratory is sufficient.

The network laboratories are classified into groups according to the complexity of the diagnostic tests performed. The main activity of the national and subnational laboratories is the analysis of IgM and immunoglobulin G (IgG) antibody in serum. Virus isolation, reverse-transcription polymerase chain reaction (RT-PCR), avidity testing, and sequencing are also available in some well-performing, strategic laboratories. Four laboratories have avidity testing and 3 have sequencing capacity. Some national laboratories also serve as reference laboratories for national laboratories in neighboring countries (Colombia/Instituto Nacional de Salud, Argentina/Laboratorio Central de Santa Fé, Caribbean Epidemiology Centre, and Panamá/Instituto Gorgas). The regional reference laboratories, in addition to performing as national laboratories, provide training to national laboratories in virus isolation and molecular characterization. Regional reference laboratories help rule out possible false-positive results and clarify indeterminate results by using additional tests. In addition to tasks performed by regional reference laboratories, the global specialized laboratory distributes proficiency panels and reagents to the network, develops and validates methods, and supports training. The global specialized laboratory is also responsible for the strain bank of measles and rubella viruses isolated in the region.

With support provided by the Centers for Disease Control and Prevention (CDC), PAHO procured test kits, general laboratory supplies, and equipment for 87% of the national measles-rubella laboratories. The subnational laboratories are supported by national governmental Ministries of Health. PAHO also provides training, support for research, advice, and oversight; facilitates quality assurance; participates in accreditation procedures; monitors laboratory performance indicators; and directs technical cooperation between Ministries of Health, PAHO, the CDC, and other partners.

AVAILABLE LABORATORY DIAGNOSTIC TESTING AND REPORTING

Infection with RV often presents as a mild rash illness, but up to 50% of infected patients may be asymptomatic [10, 11]. Thus, laboratory confirmation is essential and relies on specific RV serology (ie IgM and IgG) and/or detection of the virus.

Serology
Serology is the easiest method for diagnosis of rubella infection, due to the availability of good, commercial, RV-specific IgM and IgG tests. Enzyme immunoassays (EIA) for virus-specific IgM and IgG are the standard laboratory methods being used by the network to confirm measles virus or RV infections [12]. A recent rubella infection can be diagnosed by detection of rubella-specific IgM and seroconversion to RV-specific IgG. Suspected cases can also be confirmed if they are determined to be epidemiologically linked to a confirmed rubella case.

Rubella-specific IgM antibodies appear before IgG antibodies and can be detected after the incubation period (12–23 days). However, only about 50% of cases are IgM positive on the day of rash onset, rising to 100% after approximately 1 week. Antibody levels (IgM) usually decline to undetectable levels 6–8 weeks...
after rash onset. Because of the rate of decline of rubella-specific IgM, PAHO recommends that IgM testing be done in specimens collected no later than 30 days after rash onset. The primary difference between measles and rubella serology is the delayed rise in rubella-specific IgM and a slower decline, reaching the limit of detectability approximately 2 weeks later than measles-specific IgM [13, 14].

RV IgG antibodies appear within a week of rash onset and generally rise more slowly than IgM. IgG levels reach a peak about 2 weeks after symptoms disappear and usually persist for life. As the RV IgG response matures, the avidity of the IgG to RV increases [12, 15]. Sera of infected individuals will typically show a 4-fold or greater increase in IgG, in 2 specimens, the first collected during the acute phase of rubella (days 0–10 after rash onset) and the second during the convalescent phase (days 14–21 after rash onset, at least 7 days after collection of first specimen) [14].

Virus Isolation, Identification, and Molecular Characterization
Detection of RV or viral RNA to confirm rubella cases is a valuable diagnostic tool in support of rubella elimination. This is true since approximately twice as many rubella cases are positive for RV/RNA on the day of rash onset as are serologically positive and since, as rubella is a mild disease, follow-up visits after rash onset are difficult to obtain [16]. Both methods of detection of RV, directly in clinical specimens by amplification techniques (eg, RT-PCR) or by virus isolation, have sufficient sensitivity to be useful [16, 17]. In the Americas, 20 of 21 national laboratories have the capacity to perform virus isolation or detection by RT-PCR.

Although amplification techniques can be used to confirm rubella RNA in clinical specimens and to do limited characterization of those RNAs, virus isolation remains important because isolates provide a source of unlimited viral RNA. Unlimited viral RNA is important, since it is not yet clear whether extensive sequencing information will be necessary to identify the source of viruses of genotypes found in the Americas [17].

Obtaining significantly more virologic data in the Americas requires capacity enhancements in the laboratory network, although increased activity associated with amplifying RNAs and/or obtaining isolates for both measles and RVs in the same laboratories is currently occurring. In addition, the fact that wild-type RV infection cannot be reproducibly detected in cell culture by cytopathic effect (CPE) (eg, in Vero cells expressing human signaling lymphocyte activation molecule (hSLAM) means that additional techniques must be used to identify RV isolates [17–19]. (Wild-type measles virus infection usually produces CPE in Vero/hSLAM cells.) Detecting RV-infected cells can be done by detection of either RNA or viral antigens. A newly developed, simple, nonfluorescent antigen detection technique is useful [20].

Genetic characterization of wild-type RVs in the Americas will likely be of great benefit in characterizing rubella outbreaks and documenting rubella elimination. This was certainly the case in some countries in the Americas (eg, United States) [21]. One genotype of RV (genotype 1C) has only been found in the Americas and the elimination of this genotype should thus be tracked [22]. Virologic surveillance is important to establish a genetic baseline for indigenous RV in the Americas [23, 24]. Furthermore, genetic characterization of viral RNA is currently the only clear way to differentiate between infection with wild-type and vaccine viruses [25]. Such differentiation will become increasingly important as the number of rubella cases declines to very low levels.
To ensure that laboratory data are reliable, a quality assurance program using standard and validated assays was established in 1999. The process began with the implementation of annual proficiency test (PT) panels prepared by the global specialized laboratory. In 2005, standardized reference reagents were introduced throughout the network. Since 2006, the PT panel distributed in the PAHO measles-rubella network is the same as that distributed to measles-rubella laboratory networks in other regions, and is provided by the Victorian Infectious Diseases Reference Laboratory (VIDRL), in Melbourne, Australia. Since 2001 the results of the PT panels sent to the laboratories were in 100% concordance with expected results. National laboratories are required to ship twice a year all measles- and rubella-positive sera, all sera with indeterminate results, and up to 10% of negative sera to their respective reference laboratories. The reference laboratory then repeats the serologic tests. This process helps ensure the reliability and quality of the serological work performed by the network laboratories.

To ensure high-quality surveillance during disease elimination, PAHO monitors surveillance and laboratory performance indicators. A data management system was developed for data entry, including clinical, epidemiologic, and laboratory results. The indicators that are most relevant to the laboratory are (1) percentage of blood specimens with laboratory results within 4 days of receipt at the laboratory and (2) percentage of cases discarded by the laboratory. In 2004, new criteria to monitor laboratory performance were added, including documentation of internal quality control, timeliness and accuracy of reporting, precision of routine test results, and a site visit. The objective of the site visit, conducted every 2–3 years, is to review laboratory operating procedures and work practices [26, 27].

As rubella becomes less common, the positive predictive value of laboratory tests diminishes, increasing the chances of false positive laboratory results. The interpretation of these results is a challenge to the laboratory network and to the national authorities, particularly if the suspected case is a pregnant woman. An IgM-positive result may be due to an infection with a wild-type virus, to a recent vaccination, or to an infection with an agent that cross-reacts serologically [28–30]. To resolve possible false-positives, the laboratory network is primarily using IgM EIA in conjunction with testing for IgG increases with an IgG EIA. When the results of IgM in conjunction with IgG indirect EIA results remain inconclusive, the sera are sent to the reference laboratory in order to take advantage of expertise and specialized serologic tests available in these laboratories. In addition, wild-type RV detection (eg, virus isolation) confirms rubella; however, a negative result does not exclude rubella. The final classification of suspected rubella cases when there is little or no endemic rubella must be based on all the laboratory, clinical, and epidemiological data.

The cost of shipping, loss of specimen quality because of breaks in the cold chain, and customs delays represent major obstacles for sending specimens for confirmatory testing, virus isolation, or molecular characterization in some circumstances. The implementation of dried blood spot and oral fluid as alternative specimens could partially resolve these problems [31]. Serum and blood samples can be sent by the field to the laboratory or interchanged between laboratories by mail, and do not require the reverse cold chain during transportation. Oral fluid samples can be used to detect virus and IgM antibodies to RV and can be easily collected at first contact with a suspected case [32]. In addition, these techniques have been evaluated in the standard measles-rubella surveillance system in Peru [33]. Because an adequately functioning cold chain for sera and throat swab specimen transport already exists and because IgM testing from oral fluid requires expensive kits that are not easily available, it is anticipated that dried blood spot and oral fluid specimens will be used in special circumstances (eg, areas that are difficult to access) as rubella is eliminated.

Important challenges remain in maintaining quality and meeting the financial needs of the measles-rubella laboratory network. PAHO continues to advocate among national governments and partner agencies for sustained support of the measles-rubella laboratory network. Funding laboratory supplies for measles and rubella testing and encouraging countries to integrate these costs into national surveillance budgets are top priorities for PAHO. Laboratories should strengthen the working relationship with epidemiology staff to improve timeliness of specimen receipt by the laboratory, to adequately interpret suspected false-positive results, and to ensure that data are recorded and reported in a timely and accurate manner.

The performance indicator, specimens received in laboratory within 5 days, requires special attention (Figure 2). The low performance observed for this indicator may be explained in some cases by lack of funds for transportation from remote areas. Often, to conserve funds, health units wait to accumulate a group of specimens before transporting them to the laboratory.

Genetic data are particularly important when documenting elimination, as has been repeatedly demonstrated [33, 34]. Since the establishment of the measles and rubella elimination initiatives, the priority has been to collect specimens for serology testing, with less emphasis placed on virus isolation. The difference in the number of specimens collected for virus isolation compared with specimens collected for serology is substantial (Figures 3, 4). Figure 3 shows a considerable increase in the number of specimens collected for virus isolation from 2005 to 2007. Although a dramatic increase was observed in 2007, the number and quality of specimens collected for virus isolation is...
not yet enough to increase the RV genetic baseline, considering that in the same period only 2 genotypes of RV were detected in the region, genotypes 2B and 1j. Greater than 70% of specimens collected for serology are collected within 1–3 days after rash onset, when specimens for virus isolation should have a high probability of being positive. A much greater emphasis on collection of specimens for virus isolation in 1–3 days after onset, on high-quality specimen transport, and on good isolation techniques is needed so that a larger proportion of specimens collected for virus isolation will yield a virus. This contrasts greatly with the experience of polio elimination in the Americas, where virus isolation was a primary surveillance method.

CONCLUSION

Laboratory testing of rubella and measles cases is a critical component of surveillance. This becomes even more relevant when disease prevalence of a mild disease like rubella is very low. In these settings, each suspected case of rubella requires laboratory confirmation. The measles-rubella laboratory network established in the Americas has provided reliable information, confirming and rejecting suspected cases (Table 1), identifying circulating virus strains, tracking virus transmission pathways, and evaluating the impact of mass campaigns. The network is fully functional and the personnel are highly motivated. The consistently high percentage of cases rejected reflects the commitment of laboratories to support rubella elimination and the maintenance of measles elimination. However, our current understanding of the genetic baseline is very limited in most of the Americas and there is little time to improve our understanding of the genetic baseline for RV in the region. Such understanding will likely be critical for documenting the elimination of indigenous virus transmission, and would also help determine the sources of imported virus. Moreover, virological
surveillance should be strengthened to improve the ability to differentiate wild-type from vaccine-related infections and to discard false-positive serologic results.

The PAHO measles-rubella laboratory network has demonstrated considerable flexibility by transitioning from measles to rubella and has matured over time as required by the program’s demands. As of 2009, improved laboratory testing algorithms were being developed in order to document the elimination of rubella from the Americas. In the road ahead, these algorithms are expected to require increased use of sophisticated laboratory testing protocols (e.g., virus isolation) and increased interactions between laboratory workers and epidemiologists. More sophisticated testing will likely be developed only in select laboratories to control costs. Despite new challenges and increased responsibilities, the network continues to provide important support for the elimination of rubella and the maintenance of elimination of measles in the Americas.

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References

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