SUPPLEMENT ARTICLE

The Measles Laboratory Network in the Region of the Americas

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The success of measles eradication depends upon a laboratory network to rapidly analyze samples obtained as part of surveillance and case investigation. The Pan American Measles Laboratory Network was established in 1995. Major activities of the 22 participating laboratories include the rapid testing of serum samples to diagnose measles, analysis and recommendation of techniques to be used in serologic testing, training in virus isolation, and procurement and distribution of laboratory materials. In addition, a comprehensive quality-control program and an electronic communication network have been developed. Testing for rubella has also been incorporated. The Network has been crucial to the great progress made toward eradicating measles from the Western Hemisphere. The priority given to the laboratories in the Network must continue in order to ensure that the eradication goal is reached and that validation of the interruption of endemic transmission of measles is documented.

BACKGROUND

Building upon the success of polio eradication in the Region of the Americas, the Ministers of Health in the Americas unanimously approved the goal of measles eradication [1]. A key component to the success of this program is active surveillance and a well-trained laboratory network that can respond with rapidity and quality in the analysis of samples obtained as part of surveillance activities and case investigation. The Pan American Measles Laboratory Network was established in 1995, building upon the experience of the polio laboratory network and including the same infrastructure [2]. The major activities of the network initially included the rapid testing of serum samples to diagnose measles, the analysis and recommendation of techniques to be used in serologic testing, training in virus isolation, and the procurement and distribution of laboratory equipment and reagents. As the goal of eradication in the Americas draws closer, the network, in collaboration with local and regional epidemiologists, recently has developed a comprehensive quality-control program and an electronic communication network and has incorporated testing for rubella as part of the integrated surveillance toward the ultimate goal of eliminating both diseases from the region.

NETWORK AND OBJECTIVES

The Measles Laboratory Network consists of 22 national laboratories. Of these laboratories, 10 were chosen to function as reference centers, and three (the Centers for Disease Control and Prevention [CDC] in the United States, the Oswaldo Cruz Foundation [Fiocruz] in Brazil, and the Laboratory Center for Disease Control in Canada) were designated as specialized reference laboratories whose functions were extended to genetic sequencing and the research of new techniques

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and their application in the field. In addition, several countries (e.g., Mexico, Argentina, Colombia, and Venezuela) have developed an in-country network of laboratories that coordinate with the national laboratory. These laboratories are often geographically spread throughout the country and are important for the rapid processing of samples.

The responsibilities of the national laboratories include the reception, adequate handling, and testing of blood samples for IgM antibodies to measles and rubella virus; the accurate interpretation and reporting of test results to reporting sources; the coordination with health professionals in the distribution of viral transport media for the collection of urine, throat, and nasopharyngeal samples for virus isolation; the reception and processing (in most laboratories) of samples for virus isolation; and participation in a quality-control program consisting of proficiency panels, supervisory visits, and the regular shipment of samples to reference laboratories for quality control.

In addition to the services described above, sub-regional reference laboratories develop standards and proficiency panels for use in quality-control testing and support laboratories in the confirmation of test results and in the application of additional analytical techniques when isolated cases occur or indeterminate results are presented. Furthermore, sub-regional reference laboratories provide specialized training and field visits to improve the technical capacity of technicians and to enhance field communication.

CDC, Fiocruz, and the Gorgas Institute in Panama have provided training in virus isolation and other molecular techniques. This information is used to deduce pathways of transmission and the geographic distribution of measles virus genotypes. At the end stage, virus isolation and sequencing data are important tools when IgM-positive results are reported that may be attributed to a vaccine-related rash reaction, a possible false-positive result, or an importation from an area where measles transmission is endemic. Personnel have participated in the development of protocols, training manuals, and a Web site with a plethora of valuable technical information and links to pertinent sites.

The Pan American Health Organization (PAHO) provides leadership in the provision of test kits and other materials and equipment to countries, and it organizes sub-regional and regional meetings that bring together laboratory, expanded program on immunizations, and surveillance coordinators each year [3]. Special laboratory meetings are held to review data, quality-control issues, and proficiency panel results and to discuss new methodologies and program indicators.

LABORATORY METHODS AND REPORTING

Because clinical diagnosis alone is not sufficient to confirm a case of measles or rubella, a reliable laboratory test was needed to test serum samples obtained from suspected cases [4]. A confirmed case must have serologic confirmation or be epidemiologically linked to another confirmed measles (or rubella) case. It is recommended that a blood sample be obtained at the first contact with a suspected case [5]. Samples should be obtained within 30 days of rash onset for reliable laboratory results. This blood sample should be handled aseptically and held on ice. The sample should subsequently be centrifuged and the serum separated and delivered as quickly as possible to the appropriate laboratory for testing. A copy of the completed case investigation form should accompany each sample.

Commercial test kits based upon indirect EIA of IgM class antibodies have been validated through the Network to be both sensitive and specific [6–8]. Results can be obtained the same day. However, as the prevalence of disease declines, this method produces more false-positive results, given that it is not 100% specific [9]. When sporadic confirmed cases are reported, several other laboratory tools are available to confirm or discard EIA test results. They include an IgM antibody-capture immunoassay developed by CDC, the measurement of IgG anti-measles antibody levels in blood samples obtained from an individual at the onset of disease and at least 14 days after onset, and molecular techniques.

ISOLATION AND IDENTIFICATION OF MEASLES VIRUS IN CELL CULTURE

The availability of a sensitive cell line (B95a) for isolation of measles virus from clinical specimens and establishment of automated DNA sequencing techniques have allowed for rapid genetic characterization of a large number of wild type strains of measles virus. This database of sequence information now makes it possible to use molecular epidemiologic techniques to identify the source of wild type viruses and to differentiate between wild type and vaccine strains [10–12].

As progress is made toward the elimination of measles in many regions of the world, it will be critical to examine virus isolates from as many outbreaks and isolated cases as possible in order to identify the source of the virus. The World Health Organization (WHO) held a meeting in May 1998 to standardize the protocols for the genetic characterization of wild type measles viruses and to establish a consistent system for describing the genotypes [13]. Collection of measles specimens will help to determine which outbreaks may be related and to monitor patterns of virus transmission. The ability to determine the effectiveness of measles elimination programs will also be enhanced by continued characterization of viruses from sporadic outbreaks of measles [12].

Virus isolation and genetic characterization can take several weeks to complete. Specimens (urine, throat, or nasal) for virus isolation should be obtained at the same time that serum is

drawn because a delay in collection will reduce the chance of isolating the virus. It is recommended that samples for virus isolation be obtained within 5 days of rash onset. Urine or nasal specimens should not be substituted for serum specimens for measles diagnosis.

An Epstein-Barr virus–transformed marmoset B lymphoblastoid cell line, B95a, is the preferred cell line for primary isolation of measles virus [14]. These cells are as much as 10,000 times more sensitive than other commonly used cell lines, such as Vero and PMK, for isolation of measles virus from clinical specimens. B95a cells are relatively easy to maintain in the laboratory, and the cytopathic effect from measles infection is readily observed. However, laboratorians should note that this cell line does produce Epstein-Barr virus and should be handled as infectious material (biosafety level 2) at all times.

A flowchart indicating the order in which samples are processed in the laboratory network is illustrated in figure 1.

QUALITY CONTROL

Standardized procedures for measles laboratory quality control have been developed through the collaboration of PAHO, CDC, and the reference laboratories. These procedures are based upon standard operating procedures [15] and a supervisory checklist that is used during periodic visits to laboratories. In addition, PAHO and CDC coordinate with the Global Measles Laboratory Network in the review of draft recommendations and the participation in technical committees. Furthermore, a schedule has been established between national laboratories and their respective reference laboratories for the regular shipment of 10% of measles IgM-positive samples, all samples with indeterminate results (at the borderline in ELISA readings), and, when possible, 10% of samples of all reported dengue cases with rash and fever. The results are compared between both laboratories, and any discrepancies are evaluated.

Panels of coded specimens are prepared at CDC and the Gorgas Institute and sent to all national laboratories for testing with the EIA kits that are routinely used by countries. These panels include serum of IgM-positive measles cases, IgM-negative measles cases at several dilutions, and a few specimens with IgM measles antibody levels that should fall at the borderline between positive and negative (indeterminate). Standardized reporting forms are sent with the panels, and results from the laboratories are sent back to CDC or Gorgas for evaluation. Laboratories with results discrepant from those expected are prioritized for supervisory visits, and measures are taken to improve performance.

REPORTING AND DATA MANAGEMENT

A data management system has been developed for the entry of surveillance data, including clinical, epidemiologic, and laboratory results. The Measles Surveillance System is used in most countries in the region and has been installed in laboratories in the Network. Key indicators that are pertinent to the performance of the laboratories include the percentage of reported cases with an adequate blood sample (obtained within 30 days of rash onset); the percentage of reported cases with blood samples delivered to the appropriate laboratory in <6 days from the date the sample was obtained; the percentage of blood samples with laboratory results reported within 4 days of receipt at the laboratory; and the proportion of reported cases discarded by the laboratory. The latter indicator is calculated with the numerator being suspected measles cases that are discarded due to either a positive laboratory result for rubella, dengue, or other illness (including epidemiologically linked cases) or a negative measles result and with the denominator being all discarded cases. These indicators are analyzed and published weekly in the Measles Weekly Bulletin of PAHO. When any indicator level falls below 80%, the reasons for sub-optimal

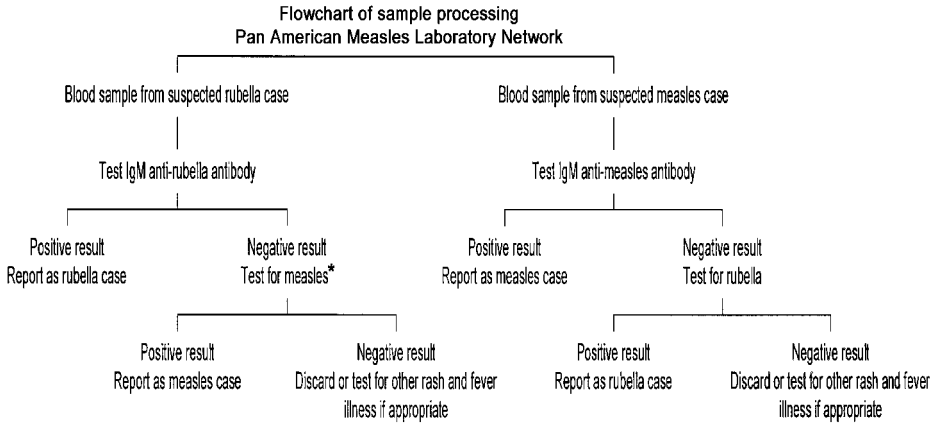

Figure 1. Flowchart of sample processing by the Pan American Measles Laboratory Network. *, In Brazil, differential diagnosis is used to determine whether to test for rubella antibodies.

performance should be discerned and corrective measures taken.

RESULTS

Table 1 includes the number of samples processed by the Pan American Measles Laboratory Network from 1999 through 2000. Specimens were tested for rubella and measles IgM serology.

Proficiency panels were sent to 15 countries in 1999–2001. Delays and problems with shipping made it necessary to repeat the sending of the panels to several countries. In all years, results sent from the laboratories were in 100% concordance with those expected, with the exception of two laboratories in 2001. Several national laboratories have begun sending their system of local laboratories panels that they prepare. These results are being gathered.

LABORATORY INDICATORS

Laboratory indicators for the region are presented in figures 2 and 3. Overall results indicate that in many cases, blood specimens are not arriving in the laboratory within the recommended time period of <6 days. There was an improvement in the year 2000, but in 2001 <60% of samples reached the laboratory within 5 days. Reasons for less-than-adequate performance include laboratories waiting to accumulate a group of specimens before testing, apathy among certain health care workers, and lack of regular transport from remote locations. However, the laboratories in the Network made great strides in assuring that once samples arrive, they are rapidly processed. From 1996 through 2001, the percentage of samples with results reported within 4 days rose from an average of 38% to 80% of samples. This impressive improvement can be attributed to a highly motivated group of technical personnel throughout the network and to the nearly constant supply of laboratory kits and material from PAHO and CDC to the network. The percentage of suspected measles cases discarded on the basis of definitive laboratory evidence has remained above 90% throughout the past 6 years, indicating that both the quality and coverage of the laboratories have remained consistently high.

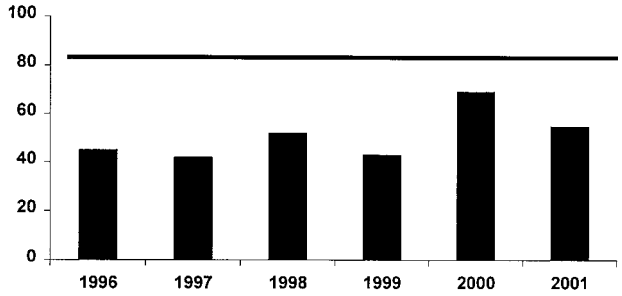

Figure 2. Percentage of laboratory specimens received in <6 days, Region of the Americas, 1996–2001. Horizontal rule represents the program goal of having ⩾80% of blood samples from laboratories arrive in <5 days. Data are as of 6 October 2001.

CHALLENGES

Communication. As with all international agencies, efficient communication among players in the administrative and operational arenas continues to be a challenge both within countries and throughout the network of laboratories. The laboratory network does not stand alone: The epidemiologists, administrative officials, and national/headquarter staff are all important to the smooth operation of the network. Within countries, methods of collecting and disseminating information vary, and the feedback mechanisms available between the laboratory staff and those managing the suspected case database and investigating the cases need to be evaluated and reviewed to determine if improvements could be made. Internet e-mail access for laboratory personnel is generally acceptable and is more reliable than overtaxed telephone/fax lines, but interruptions in service and frequent changes in addresses are also common.

Limitations of diagnostic tests. Surveillance for measles cases may capture other rash illnesses, particularly when there are few or no cases of measles circulating in a population. The available immunoassays for detecting IgM to measles have very high specificity and sensitivity [6], but such tests will occasionally result in false-positive reactions [9]. In addition, an IgM-positive result can occur for at least 6 weeks following vaccination, and in some instances, IgM can be detected >2 months after vaccination. A person presenting with rash >2 weeks after vaccination may be a true case or have rash due to some other cause. The presence of IgM in the serum will

Table 1. Number of specimens processed by the Pan American Measles Laboratory Network, 1999–2000.

Year	Total no. of samples processed	No. of IgM-positive measles samples	No. of IgM-positive rubella samples	No. of samples discarded	No. of vaccine-associated cases
1999	41,835	2605	5106	34,124	46
2000	46,886	852	5323	40,711	38

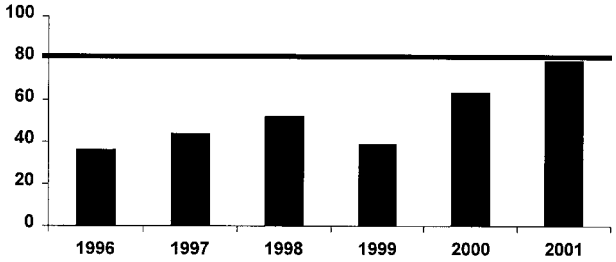

Figure 3. Percentage of serum samples processed in ≤4 days, Region of the Americas, 1996–2001. Data are as of 6 October 2001.

not be helpful since one cannot distinguish between IgM response from vaccination and wild measles infection.

Collection and transport of samples. There must be adequately trained staff available to travel to the location of the suspected cases of measles or rubella and to initiate the steps required to collect and oversee the shipment of samples. Serum specimens for antibody testing and urine or respiratory specimens for viral analysis are required. Often, the serum is collected but the specimen for viral analysis is not obtained.

The proper storage conditions and timely arrival at the laboratory for both serum and urine or respiratory samples from the patient involve logistical challenges at one or more levels. Local shipping of samples may be hampered by difficult terrain or lack of regularly scheduled transport services. Sample quality can be adversely affected by delays in shipment or when refrigeration methods are inadequate. Intracountry shipment of serum samples from local laboratories for confirmatory testing or of samples for virus isolation sent to national, regional, or reference laboratories may require courier services or airline transport. Shipment of biological specimens is becoming increasingly problematic as both methods of transport have become reluctant to accept such materials, citing safety concerns. For international shipments to reference laboratories, there are additional difficulties associated with country clearance or regulatory delays.

Training and laboratory support for quality control. The evaluation of training and other laboratory needs should be done in a timely fashion in order to plan workshops and set up funding for meeting those needs. On-site visits and periodic correspondence to laboratories and discussions with the staff may identify needs and suggest interventions for improving quality control and for addressing supply issues or staffing requirements.

The ability to transport supplies to the areas where they are needed is hampered by the same logistical obstacles discussed above. In addition, customs-related delays can damage temperature-sensitive supplies during shipment. Administrative costs to ship goods are considerable, and shipments requiring dry ice for >2 days in transit generally require specialized courier services.

Throughout the Network, there is a diminishing supply of measles and rubella IgM-positive serum, which is needed for preparation of proficiency panels and for inclusion as internal positive controls in the immunoassays. In addition, laboratory equipment requires periodic maintenance and calibration in order to assure proper standards of performance. The availability and funding of such services may be difficult to acquire in some areas.

SUMMARY OF PROGRESS MADE

Improvements in communication between epidemiology and laboratory staff and among network laboratories are being seen in many member countries. Reporting units within a national laboratory's network can benefit through use of standardized communication. Feedback can stimulate reporting and also pinpoint problem areas and needs. In Argentina, weekly bulletins have been established among the 22 laboratories in the network. Internet resources have also been useful in providing a channel for feedback. Chile operates a Web site that is updated with measles surveillance data, and a global measles laboratory network Web site, operated from the CDC, has been created to facilitate communication and rapidly share measles genotype information. A contact list is planned for the Web site so that laboratory and administrative staff can update their address lists and promptly locate contact information.

Measles and rubella surveillance has improved the diagnostic capabilities by enabling laboratories to test for either agent when the primary suspected etiologic agent is ruled out. Inclusion of suspected rubella cases has also improved the surveillance for measles. With more non-cases of measles being picked up by surveillance, additional guidance for interpretation of test results and clear definitions for ruling out cases have been prepared. The epidemiologic investigation triggered by a suspected case of measles has the effect of stimulating local staff to assess coverage and keep vigilant for any measles activity. For possible vaccine-related cases, the ability to differentiate wild from vaccine virus underscores the importance of collecting specimens for virus isolation.

Coordination between laboratory staff and program managers in the network is underway to prepare shipments and share information regarding the recommended methods of sample shipment and the proper packaging for shipping biologic samples. Advance notice of shipments and proper paperwork should expedite the process of shipping samples and supplies.

Overall, the Pan American Measles Laboratory Network has been a vital component in the great strides made toward the eradication of measles from the Western Hemisphere. The dedication and priority given to the laboratories in the Network must continue in order to ensure that the goal is reached and

that validation of the interruption in endemic transmission of measles is documented through the highest quality surveillance system.

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