

Development of the Global Measles Laboratory Network

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The routine reporting of suspected measles cases and laboratory testing of samples from these cases is the backbone of measles surveillance. The Global Measles Laboratory Network (GMLN) has developed standards for laboratory confirmation of measles and provides training resources for staff of network laboratories, reference materials and expertise for the development and quality control of testing procedures, and accurate information for the Measles Mortality Reduction and Regional Elimination Initiative. The GMLN was developed along the lines of the successful Global Polio Laboratory Network, and much of the polio laboratory infrastructure was utilized for measles. The GMLN has developed as countries focus on measles control activities following successful eradication of polio. Currently more than 100 laboratories are part of the global network and follow standardized testing and reporting procedures. A comprehensive laboratory accreditation process will be introduced in 2002 with six quality assurance and performance indicators.

ROLE OF THE LABORATORY IN MEASLES SURVEILLANCE

The laboratory plays an increasingly important role in measles surveillance as the level of disease control increases. Recognition of potential measles cases is based on clinical case definition [1]; however, it is well established that clinical diagnosis is inaccurate during the elimination phase [2] and that laboratory confirmation of suspected cases, complimented by genotyping of circulating measles strains, is critical for effective surveillance. The laboratory has two functions in measles surveillance: The first and most important is monitoring and verifying virus circulation. The second is assisting

in determination of the measles susceptibility profile of a population in specific situations.

Monitoring and verifying virus transmission.

During early stages of an outbreak, the laboratory confirms the clinical diagnosis of suspected cases. During the elimination phase, the laboratory confirms or discards any suspected cases of measles. The laboratory identifies measles virus strains and genetically characterizes measles virus from each chain of transmission.

Determining a population's susceptibility profile.

In order that immunization campaigns may be assessed, the age distribution of measles susceptibility can be determined by one of three methods: assessment of the age distribution of cases, analysis of vaccination coverage profiles over time, or by laboratory assessment of population immunity by looking at protective levels of antibody in representative subsets of the population. For each sequential phase of the measles control initiative, there are specific laboratory surveillance activities (table 1). In performing its functions, a laboratory cannot act in isolation but must be organized into a supportive network that will efficiently provide accurate information to the initiative.

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Table 1. Role of the laboratory in measles mortality reduction and elimination.

Phase of measles control, strategy	Laboratory function
Mortality reduction	
Improve routine immunization coverage to $\geq 90\%$ with 1 dose; provide 2nd opportunity for measles immunization through routine or supplementary activity	Confirm initial cases during outbreaks; determine genotypic data on circulating viruses
Establish measles surveillance	
Measles elimination	
Maintain very high routine immunization coverage ($\geq 95\%$ with 1st dose); provide 2nd vaccination opportunity	Confirm clinical diagnosis of suspected cases to help in early detection of virus circulation; analyze wild virus strains from selected cases to identify virus strains circulating and to complete global genome mapping of measles virus; in special circumstances, establish seroprevalence of the population and assist outbreak forecasting and evaluate impact of mass campaigns
Establish case-based surveillance	

THE GLOBAL LABORATORY NETWORK FOR MEASLES SURVEILLANCE

There are five main objectives for establishing a network of laboratories that supports various aspects of measles control. First is the development of standards and quality control for the laboratory confirmation of measles and provision of the necessary support as the initiative evolves. Second is the need for mechanisms for reference and support for regional and national laboratories in the diagnosis of measles and other rash illness. Third, the laboratory provides training resources and facilities for staff of regional and national laboratories. Fourth, the laboratory is a source of reference materials and expertise for the development and quality control of improved diagnostic tests, and fifth it serves as a repository for measles virus isolates for molecular epidemiology and reference sera for quality control.

Individual laboratories will not be expected to undertake the full range of tasks but will perform specific duties according to national and regional needs. Laboratories in the network are monitored by regular proficiency testing, by evaluation of performance indicators, and starting in 2002, by a comprehensive accreditation process.

Several infections cause rash-fever disease that is commonly mistaken for measles [3, 4]. The introduction of tests for rubella, dengue, and parvovirus B19 into network laboratories will increase the need for the identification of the cause of illness by public health teams and individual clinicians to avoid unnecessary public health intervention.

Measles-mumps-rubella or measles-rubella vaccines are used in many countries. Surveillance for rubella is based on clinical rash/fever surveillance, which like that for measles is inaccurate. Cases in the elimination phase require confirmation by the detection of specific IgM in the laboratory. The measles laboratory network will provide diagnostic support for rubella

control programs where needed because of the synergies for clinical surveillance and laboratory confirmation.

STRUCTURE AND FUNCTION OF THE GLOBAL NETWORK

The Global Measles Laboratory Network (GMLN) is based on the successful model of the Polio Laboratory Network. Development of the polio network began in 1986 in the Americas [5] and by 2001 encompassed 147 laboratories globally. All of these laboratories follow standardized laboratory testing and reporting procedures and use standardized reagents and equipment. To ensure quality of results and timeliness of reporting, all laboratories are expected to meet seven quality indicators that are continuously monitored. Each laboratory undergoes an annual independent accreditation assessment, which usually includes a site visit. The accreditation process began in 1998 and by mid-2002 every country in the world was served by an accredited polio laboratory.

The GMLN is still in its expansion phase, developing as countries achieve polio eradication and reallocate resources and focus activities on accelerated measles control. It currently comprises more than 100 laboratories (figure 1), an increase from 60 in 2000, and should have several hundred laboratories by 2005. The GMLN has exploited the capital investment and infrastructure development of the polio network by using many of the same laboratories for measles. Polio diagnosis is based on a cell culture-based procedure and although measles case confirmation is primarily based on serology, many of the polio laboratories have the capability and capacity for serologic testing. When isolation of measles viruses is warranted, the cell culture experience of the polio laboratories is invaluable. In some countries measles case confirmation may be done in a laboratory separate from the poliovirus laboratory that may be in the same institution, allowing full utilization of the infra-

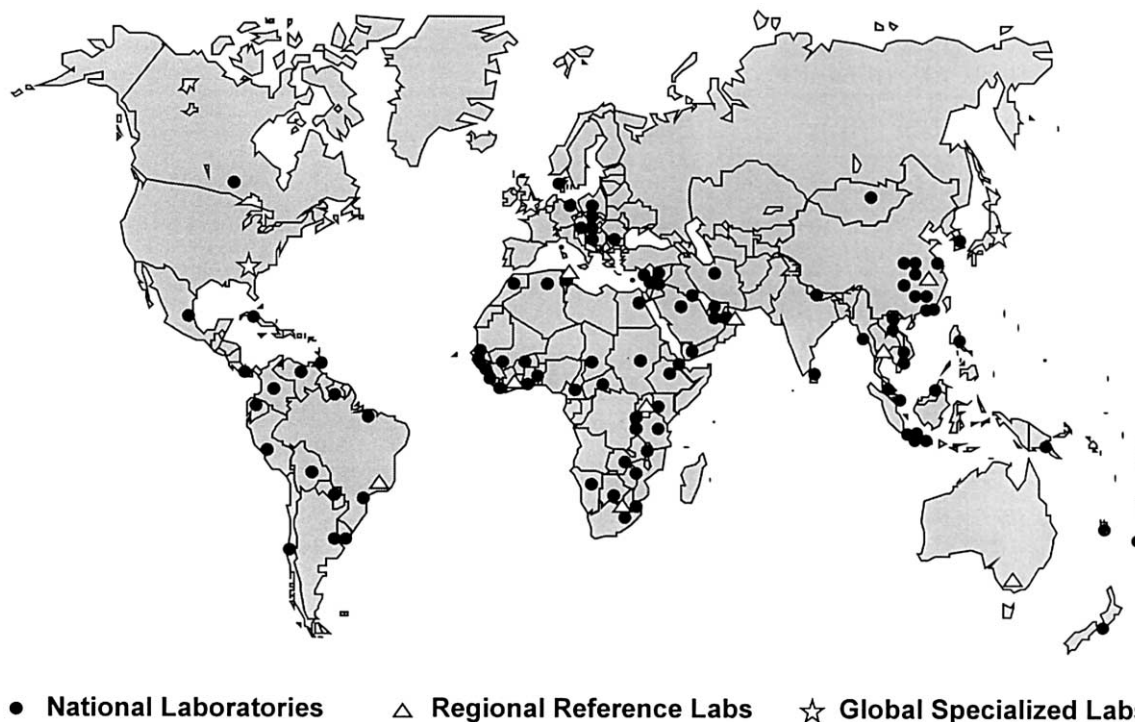


Figure 1. Measles Laboratory Network, September 2002. Dotted lines suggest approximate borders for which there may not be full agreement. The designations used and the presentation of material do not imply the expression of any opinion on the part of the WHO secretariat concerning the legal status of any country, city, or area of its authorities or concerning the delimitation of its frontiers or boundaries.

structure and communication links with the disease surveillance program and ministries of health established during the polio eradication initiative.

The GMLN will be more extensive than the polio laboratory network due to the anticipated greater number of samples to be tested per country and may exceed the capacity of a single laboratory. Also, because of transportation logistics and capacity needs, some countries will require more than one laboratory. Several countries with no previous global laboratory network experience will establish laboratories as part of the measles laboratory network. It is essential that the laboratory network be planned in tandem with regional control and elimination programs. In several countries, laboratories were established for up to 12 months before surveillance activities developed to the extent that samples were sent to the laboratory on a regular basis. A regular flow of samples from suspected cases is essential to maintain laboratory skills.

The GMLN is being organized on four levels: global specialized, regional reference, national, and subnational laboratories. Global specialized laboratories set the technical standards for laboratory diagnosis. Their responsibilities extend to measles laboratories in all regions and countries.

Regional reference laboratories are “centers of excellence” in each region and can undertake international responsibilities. They serve as reference laboratories for national laboratories

in neighboring countries and as national laboratories in their own countries. Each WHO region may have three or four regional reference laboratories.

National laboratories will have the closest links with national immunization and surveillance staff. They will test specimens from suspected cases by IgM ELISA and report directly to the staff in charge of measles control. The number of national laboratories and the order in which they are established will depend on epidemiologic priorities and available resources.

Subnational laboratories may be established in countries where, due to the large population size, testing of specimens for measles may be beyond the capacity of a single national laboratory. These laboratories may be established at provincial or prefecture levels. Geographic barriers may also present transportation difficulties for sending samples to a single laboratory and require the establishment of a second laboratory within a country.

The rate of network development is governed by four considerations: meeting the needs of the measles control initiative, monitoring laboratory performance and reliability, meeting the human and financial resources required to establish and maintain the network, and monitoring progress in achieving polio eradication goals.

Laboratories are selected after preliminary surveys of a facility’s existing virologic capacity and capability. Laboratories

are assessed on their proven capability to perform testing and whether they have appropriately trained scientists and technicians, adequate facilities, good management practices, adequate resources to cover running costs, and suitable equipment to conduct routine serologic assays. Potential laboratories are nominated in consultation with the national authority with which they would be working in order to ensure local (country) network ownership. Efforts are continuously made at the international level by the World Health Organization (WHO) and other partner agencies to generate resources to meet any financial and human shortfalls of the network.

STANDARDIZATION OF TECHNIQUES

The primary activity of the measles laboratory is the confirmation of suspected measles cases by detecting the presence of measles-specific IgM antibody in serum. A secondary but equally crucial activity is the detection of measles virus from urine or nasopharyngeal samples for genomic analysis and comparison with known strains, providing information on likely origin of the virus and transmission history.

IgM ELISA. The WHO Measles Laboratory Network selected the IgM ELISA as the standard method for confirming measles cases. Measles-specific IgM appears within the first few days after onset of rash and declines rapidly. It is usually not detectable after 4 weeks. A number of commercial IgM ELISAs have been independently assessed and found to have good sensitivity and specificity when tested on panels of sera from confirmed measles cases [6]. IgM levels are low in the first 3 or 4 days after rash onset and assays that have a high sensitivity for detecting IgM during this period are recommended for use in network laboratories.

Measles IgM ELISAs are commonly based on one of two formats—a capture ELISA or an indirect IgM detection procedure, where potentially interfering IgG antibody is adsorbed before testing. Commercial assays in both formats have good sensitivity and specificity [6]. Most ELISAs are easy to perform, require only small volumes of serum (10–50 μ L), and can be completed in less than 4 h. Required reagents in addition to those provided in the commercial kits are readily available in most laboratories. The equipment for performing and reading the ELISA is common to several other serologic tests (e.g., human immunodeficiency virus and hepatitis B) and is usually in place in most countries.

Some countries have chosen to use “in-house” IgM ELISAs, produced either in their own laboratories or elsewhere in the country. Others have selected nonvalidated commercial assays, where the prime selection criteria appear to be price. In such instances, assays should be validated by use of standardized serum panels and documentation on routine quality assurance

of production should be provided before being used for routine measles surveillance.

Measles isolation. Measles virus is excreted from infected cases only for the first 5–7 days after rash onset, often in low titer, necessitating a laboratory with cell culture or polymerase chain reaction (PCR) expertise to detect the virus. For these reasons, attempts to detect virus from suspected measles cases is not considered to be a useful diagnostic tool. However, the detection of measles virus, subsequent genomic analysis, and the availability of an extensive sequence database for wild type measles viruses have enabled molecular epidemiologic studies of measles. These studies have significantly contributed to measles control efforts by enabling investigators to identify the source and transmission pathways of the virus [7].

Unlike poliovirus, which undergoes mutations of the genome at a relatively constant rate of 2% per year, the measles genome is relatively stable and shows minor detectable changes over the course of an outbreak or even over 12 months. Hence, isolation of virus from all cases, as is attempted with polio, is not considered necessary and 1 or 2 isolates from each outbreak or chain of infection will provide sufficient data to determine transmission pathways. Although the detection and genomic analysis of measles virus is essential, few samples will be needed for this purpose, and laboratories with established cell culture or PCR facilities will do virus detection. Laboratories without virus isolation capabilities will process the appropriate samples and transport them to a nominated isolation laboratory. Isolation laboratories will in turn forward any viruses identified to the global specialized laboratories for genomic analysis.

Collection and recording of samples. The correct type and timing of specimen collection with respect to clinical signs and patient history is crucial for interpreting results and an accurate conclusion. It is recommended that a single blood sample for IgM detection be collected at the first contact with the health care system. However, because low levels of IgM are produced in the first 3 days after rash onset, about 30% of cases may be missed with the IgM assay [8]. The number of samples collected for case confirmation is dependent on each country’s phase of measles control. During the mortality reduction phase when measles is likely to be endemic, only outbreak confirmation is required to prevent overloading the laboratory. Serum samples collected from the first 5–10 cases in each outbreak should be adequate to confirm the cause. Genomic analysis of samples taken for isolation from these cases can determine whether confirmed cases are due to imported or to indigenous measles virus. Urine, nasopharyngeal, or peripheral blood lymphocyte samples for measles virus detection should also be collected from about 10 cases to ensure 1 or 2 isolates.

In the elimination phase when measles is likely to be rare, every suspected case should be investigated and serum samples should be collected for measles IgM testing. Because the positive

predictive value of confirming cases by IgM serology may be low in this phase, with most positives being false positives, some countries have elected also to test samples for measles virus to increase the sensitivity of case detection. If an outbreak occurs in this elimination phase, to ease the workload of the laboratory, only serum and isolation samples from 5 to 10 cases at the start and the end of the outbreak need be collected.

QUALITY ASSURANCE

The assurance of high technical quality and consistent performance in many widely dispersed laboratories is addressed in several ways. These include a WHO manual for the laboratory diagnosis of measles infection [9], comprehensive training courses, regular proficiency testing, an accreditation program, and validated assays.

Laboratory manual. The WHO laboratory manual outlines the disease process, discusses phases of measles control, and specifies the role and function of the laboratory network. There is detailed information on sample collection, transportation, storage and testing, data management, and reporting procedures. The manual was published in English in 1999 [9] and has been translated into French [10], Chinese, and Russian.

Training. Since 1997, 13 formal training courses involving more than 170 trainees have been held globally. Some courses specifically focus on measles, but most make use of the opportunity to integrate training on rubella surveillance and other diseases appropriate to the region such as yellow fever in western and central Africa and dengue in some South American countries. The courses provide training in standard techniques and emphasize the need for quality assurance, reporting mechanisms, and the role of the laboratory network in the measles control initiative. After formal training is completed, trainees are expected to complete a proficiency test panel on return to their laboratories. Follow-up site visits to the trainee's laboratory by specialist virologists will occur if the proficiency test results indicate problems.

Standardized reagents and cell lines. After assessment with panels of validated sera, several commercial ELISAs with good specificity and sensitivity have been recommended for use in the GMLN. The sensitivity and specificity of these assays together with the capability of the laboratory technicians is monitored by referral and retesting of a proportion of samples by the regional reference or global specialized laboratories. Several global specialized laboratories have collected panels of validated sera for assessing and quantifying measles IgM assays and for the development of proficiency panels. B95a, a highly sensitive marmoset lymphoblastoid cell line [11], is currently recommended for isolation of measles virus. A small number of global specialized laboratories have repositories of these cells

for distribution to measles network laboratories performing measles isolation.

Meetings. Regular subregional, regional, and global meetings are essential to allow development and the smooth running of the network. Regular contact between the laboratory and the national and regional measles programs is critical for reviewing progress, developing testing strategies, considering new techniques or methodologies, monitoring quality, and in general to strengthen the network concept.

Proficiency testing. An ongoing proficiency testing program has been established. Laboratories are provided with a comprehensive coded panel of sera containing IgM to a wide range of rash-causing illnesses and negative sera. Proficiency test panels are distributed to laboratories at the completion of training courses and to all network laboratories at least annually. In 2001, all but 2 of 46 laboratories (mostly newly established laboratories in the WHO African and Eastern Mediterranean regions) had passing scores of >90%.

Accreditation. The assurance of high technical quality and consistent performance for a large number of laboratories will be addressed by establishing an accreditation program based on that created for the polio laboratory network. A relevant WHO regional office will annually accredit national measles laboratories on the basis of laboratory performance in the immediately preceding 12 months. Beginning in 2002, all laboratories will be assessed annually for six performance and quality-related criteria, including a site visit if necessary. The criteria will include proficiency testing performance, documentation of internal quality control measures undertaken, timeliness and accuracy of reporting, and precision of routine test results. On-site there will be a comprehensive review of laboratory activities including laboratory management, communication and cooperation with the measles program, adequacy of equipment and staffing, and biosafety awareness. The primary aim of the accreditation process is to ensure that laboratories achieve an objectively assessed high level of performance. Laboratories that fail to meet any of the criteria will be fully supported to meet accreditation standard as rapidly as possible.

MONITORING AND COMMUNICATION

The ability of the measles control initiative to respond rapidly and effectively to evidence of measles transmission is dependent on timely and accurate laboratory information. The laboratory is required to report results to national authorities in charge of measles control within 7 days of receiving samples. The completeness and accuracy of information received with the specimen determines how effectively virologists can provide programmatically meaningful results. For this reason a minimum set of data is recommended for collection on specimen request forms that is collated into a laboratory specimen da-

tabase [12]. A computerized data management system is being developed that will permit rapid access to laboratory data and assist in reporting summary or case-based data.

THE FUTURE

Alternative sampling methods to serum collected from venipuncture have been investigated, and some have equivalent sensitivity and specificity for detecting measles IgM approaching those of serum. Oral fluid samples have been successfully used as the principal specimen for measles diagnosis in the United Kingdom for more than 5 years [13]. This noninvasive sampling procedure requires minimal training and avoids the risk of needlestick. Measles virus genomic material may also be detected in oral fluids if collected within the first week of rash.

Dried whole blood spots collected on filter paper have been used for many years, especially for IgG determination. IgM can also be detected from dried blood spots with only slightly lower sensitivity and specificity than with serum, 95% and 96%, respectively [14]. Recent findings from the Netherlands indicate that measles nucleic acid is sufficiently stable in dried blood to allow detection of measles virus by PCR, even after storage for 25 weeks at 25°C [15]. The attraction of dried blood spots is the ease of specimen transport from the field to the laboratory without need for a reverse cold chain during transportation. Field evaluation of both dried blood and oral fluid collection methods for possible network use is underway.

CONCLUSION

Good progress has been made in establishing and developing a GMLN. Challenges facing the GMLN include securing sufficient funding to develop and maintain the network, establishing an appropriate and acceptable quality assurance mechanism, and resolving how to effectively accommodate laboratory testing for other rash and fever illnesses (e.g., rubella and dengue). Development of the measles laboratory network to date has made use of facilities and resources provided by the polio network, a strategy envisioned in the original goals of the polio laboratory network. There are several constraints upon the GMLN that should be overcome with effective plan-

ning and good management. The network is developing rapidly and strategies for further development and expansion have been established. With adequate international support, this network will provide crucial information for measles control activities and the eventual eradication of measles.

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