Poliovirus Surveillance: Building the Global Polio Laboratory Network

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A network of virologic laboratories has been established by the World Health Organization to conduct surveillance for wild poliovirus and to provide evidence for the certification of poliomyelitis eradication. The network consists of >60 national laboratories isolating and identifying polioviruses within countries; 16 regional reference laboratories, providing intratypic differentiation of wild and vaccine strains and assisting with quality assurance and training; and 6 global specialized laboratories, conducting research, preparing reference reagents, and providing genomic sequencing of wild polioviruses, advanced training, and expert virologic advice. Laboratories collaborate with national eradication programs in the detection, reporting, clinical investigation, and virologic testing of stool specimens obtained in connection with cases of acute flaccid paralysis and, where indicated, from healthy children and the environment. A quality assurance system, leading to World Health Organization accreditation, involves training in standardized techniques, use of centrally prepared typing antisera, annual proficiency testing and follow-up action, and monitoring of standard performance indicators.

Poliomyelitis surveillance provides the information for programmatic action. It serves as a method for assessing the effectiveness of immunization strategies and as a tool for guiding efforts toward eradication of the disease and the virus. Surveillance is based on the detection, reporting, clinical investigation, and virologic testing of stool specimens from all patients with acute flaccid paralysis (AFP). Successful surveillance depends on the cooperation of public health workers, clinicians, epidemiologists, and virologists. Poliovirus surveillance provides the evidence for certification of eradication. Surveillance systems of proven sensitivity are required to document the absence of wild virus isolations from AFP patients, healthy children, and the environment. Key to poliovirus eradication and its certification is a global network of high-quality poliovirus laboratories capable of detecting wild virus when and where it occurs. Building the network began in 1986 in the Americas, the first region to declare its intention to eradicate poliomyelitis, and has continued in the other regions of the World Health Organization (WHO), following the global resolution of 1988 [1].

Structure and Functions of the Network

The polio network includes laboratories with a wide range of capabilities and capacities designed to complement each other and to provide appropriate services to countries at different phases in their eradication programs. The network consists of national laboratories, regional reference laboratories, and specialized laboratories [2].

The national laboratory, of which there are >60, is the heart of the system, working closely with clinicians, epidemiologists, and Expanded Programme on Immunization (EPI) managers to investigate cases of AFP and to detect and identify polioviruses in stool samples. Emphasis is placed on the use of basic, sensitive techniques for poliovirus isolation and identification and rapid reporting. These laboratories also perform programmatically essential serologic or stool surveys and test oral polio vaccine potency if breaks in the cold chain are suspected. Strains of poliovirus isolated by the national laboratories are referred to regional reference laboratories for determination of whether they are wild or vaccine in origin.

The 16 regional reference laboratories are located in the Central African Republic, Ghana, and South Africa in the African Region; Egypt, Pakistan, and Tunisia in the Eastern Mediterranean; France, Finland, the Netherlands, and Russia in the European Region; India, Sri Lanka, and Thailand in the South-East Asian Region; and Australia, China, and Japan in the Western Pacific Region. In the American Region, where poliovirus eradication has been certified, the network of poliovirus laboratories continues to serve a sentinel role.

The regional reference laboratories have a dual role. They provide basic services for their own countries and for those countries in the region that do not have laboratories. In addition, they support designated national laboratories in training and quality assurance by hosting formal training courses, accepting virologists for specialized training, or visiting countries to provide on-site instruction. The regional reference laboratories receive isolates for confirmatory testing and intratypic differentiation, distribute reference materials such as antisera and cell cultures, and coordinate proficiency testing. These laboratories provide a crucial link between the specialized and national laboratories by assisting in the development and field testing of new technologies.
Unlike the national and regional laboratories, which are grouped within the WHO regions, the activities of the 6 specialized laboratories, located in Atlanta, Bilthoven (Netherlands), Helsinki, London, Paris, and Tokyo, are global in scope. These laboratories prepare the reference reagents and proficiency panels of unknown viruses on which the quality control system is based. They develop training materials and offer advanced training on the molecular and antigenic characterization of polioviruses. They provide genomic sequencing for the characterization of wild viruses as to time and geographic location. The specialized laboratories also conduct research on methods to improve the sensitivity and specificity of virus detection. Their staff members assess network laboratories and provide expert advice on virologic and programmatic issues.

Selection of Laboratories

The size of the network is governed by two considerations: the need for global coverage and the need to closely monitor the laboratories to assure performance quality and reliability. The objective is to enlist the minimum number of laboratories required to process the projected number of specimens from AFP cases in all countries, based on the expected occurrence of 1 case per 100,000 population <15 years old.

In each region, preliminary surveys were done of existing virologic capability and capacity. Potential members of the network were nominated in consultation with the national authorities with whom they would be working to ensure ownership of the network by the countries. The majority of virology laboratories are affiliated with either governmental or university institutions, thus encouraging national responsibility for the basic facility and staff and assuring lasting benefit to the country of any upgrading of facilities, training, or introduction of new technologies. Ideally, the poliovirus laboratory in many countries and regions will serve as the model for surveillance of other diseases of public health importance.

Laboratories become accredited members of the WHO polio network through a process of nomination, training, testing in basic proficiency, and formal agreement of the national health authorities.

Standardization of Techniques

The primary activities of the poliovirus laboratory are virus detection and characterization. Other activities, such as sera surveys and vaccine potency testing, also have important places in the eradication initiative and have been included in training and applied where appropriate. For each of these, standard procedures are specified.

Virus isolation and identification. Classical cell culture methods recommended for the network are based on those described by Kappenburg [3]. The continuous cell lines Hep-2 and RD are used because of their availability, ease of maintenance, and proven sensitivity to infection by polio and other enteroviruses. Cell stocks are centrally produced and distributed throughout the network via regional reference laboratories. Basic isolation procedures consist of treating fecal specimens with chloroform and observing cell cultures for two passages of 7–10 days before they are reported as negative. Isolates are identified by microneutralization tests using pools of rabbit antisera produced by one of the specialized laboratories at the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in the Netherlands. These monospecific typing antisera have homologous titers in excess of 40,000 and heterologous titers of <10, thus permitting the identification of viruses present in low concentrations in mixtures. Enteroviruses are identified using pooled antisera from RIVM or the Lim-Beynish Melnick pools.

Intratypic differentiation of polioviruses. The disappearance of wild polioviruses is documented by determining whether isolates are wild or vaccine-derived. Accurate intratypic differentiation through the use of relatively simple and reproducible assays becomes more important as countries approach eradication. Two methods are recommended to the regional reference laboratories on the basis of a collaborative study among the specialized laboratories [4].

One method is based on the antigenic differences between wild and vaccine strains as detected by polyclonal cross-absorbed antisera in an ELISA. The other method is nucleic acid hybridization, using RNA probes against the Sabin vaccine strains and the wild strains endemic to the region.

Poliovirus antibody assay. Poliovirus sera surveys can provide useful information on population susceptibility. A standardized microneutralization method is recommended for laboratories in the WHO network [5]. Antibody assays are not recommended for routine use in the diagnosis of poliomyelitis, because increases in serum antibody titers in an environment of widespread immunization are difficult to interpret. Further, there are no tests that distinguish between antibodies stimulated by wild or vaccine strains.

Potency testing of oral polio vaccine. The assurance of vaccine potency is the responsibility of the national control authority in each country. Polio network laboratories may be called upon from time to time to titrate oral polio vaccine following interruptions in the cold chain. A standard WHO method is available for titrating individual virus types [6], but network laboratories are requested to determine only total titers, since all three types in the vaccine are expected to deteriorate at the same rate under adverse conditions.

Genetic characterization of polioviruses. Advantage has been taken of the constant rate of genetic mutation of wild polioviruses to track transmission pathways through analysis of genetic relatedness of wild poliovirus strains from different countries and years [7]. Strains with a high degree of genetic homology are found to cluster geographically, and these genotypes tend to be systematically eliminated as polio eradication strategies are implemented. Genomic sequencing and analysis are largely the responsibility of the specialized laboratories. The collections of poliovirus strains that make meaningful anal-
ysis possible are the result of collaboration between all laboratories of the global network.

**Quality Assurance**

The assurance of high technical quality and consistent performance in a large number of widely dispersed laboratories is addressed by training, providing standard high-quality typing antisera and sensitive cell cultures, establishing a proficiency testing program, and offering corrective and supportive action to laboratories not meeting the required performance standards.

**Training.** Twenty formal training courses have been given over a 5-year period in all regions of WHO. The purpose of these courses for virologists and technicians is to provide initial or refresher training in the standard techniques, to inform them of the aims and strategies of the eradication program, and to create an awareness of their role in helping to maintain a strong and cohesive network of laboratories. The formal training is followed as needed by assigning persons for 2–6 weeks to regional reference or specialized laboratories and by in-country training. Training in molecular techniques is given through longer periods at appropriate reference laboratories.

**Reagents.** Standardized polio and enterovirus typing antisera are important tools for accurate and consistent identification of isolates. Standardized reagents are used in training and are provided on request to all laboratories. Probes and cross-absorbed antisera for intratypic differentiation are similarly formulated and standardized by the producing laboratories.

**Proficiency tests.** Panels of unknown viruses in stool suspensions are available in sufficient quantity to last for several years. They consist of single viruses, mixtures of polio and nonpolio enteroviruses of different titers, and negative samples. The test panels are designed to reveal flaws in technique that may compromise poliovirus detection. Proficiency testing panels are distributed at the end of each training course and serve as an initial indicator of the ability of virologists to establish the techniques in their home laboratories. Each laboratory is to be tested at least annually. A coded analysis of proficiency test results for each region is provided to all participants. A minimum score of 80% is required to maintain the laboratory in good standing within the network.

**Follow-up action.** A proficiency test score of <80% is indicative that a laboratory may be experiencing difficulty in maintaining the required standard of performance and triggers one or more responses. First, the laboratory worksheets are reviewed carefully to determine possible sources of error. If more serious problems are suspected, the laboratory may be visited by a virologist to review the methods being used, determine the needs and constraints, and recommend additional training, supplies, or equipment. The proficiency test is repeated to ensure correction of the problem(s). If the second test score is also <80%, routine laboratory results are verified by referring a proportion of original specimens and isolates to a reference laboratory for a defined period of time or until the problem is corrected. Eventually a decision is made on whether the laboratory is able to continue to support polio eradication.

**Supplies and equipment.** Key to the quality of laboratory performance is the availability of appropriate supplies and equipment. All laboratories must have functioning basic equipment such as microscopes, centrifuges, incubators, refrigerators, mechanical and nitrogen freezers, water purification units, and testing devices. Essential supplies are those required for specimen processing, cell culture maintenance, and virus identification. Meeting basic needs of laboratories in many developing countries presents serious challenges because of either inadequate national funding, high tariffs on imports, or excessive time required for approval of purchases. For many laboratories, these challenges are being met over time through WHO assistance and funding. Additional assistance is provided through the Rotary International Polio Plus Partners project, the Japan International Cooperation Agency, and contributions of other donor partners.

**Monitoring and Communication**

Laboratory performance and the quality of poliomyelitis surveillance is assessed through standard performance indicators. For AFP surveillance, performance indicators have been established for case finding, reporting, and investigation. Field investigators are required to collect 2 stool specimens, 24–48 h apart and within 14 days of onset of symptoms, from ≥80% of all reported case-patients. Stool specimens are to be sent to the laboratory within 3 days of collection at a temperature of ≤8°C.

The laboratory is required to report virus isolation results to program managers within 28 days of receipt of the specimens and to report intratypic differentiation results within 2 months. In addition to passing the annual proficiency test, laboratories are required to isolate enteroviruses from ≥10% of all specimens tested. Reports are to be forwarded by the laboratories to regional offices at weekly, monthly, or quarterly intervals, summarizing results and the extent to which the performance goals are being met.

The ability of virologists to provide programmatically meaningful results depends on the completeness of information received with the specimens. For this reason, a minimum set of data is recommended for specimen request forms. The use of unique EPI numbers to facilitate linkage of specimens with cases and contacts is strongly advocated.

A computerized data management system that integrates AFP case information and laboratory results is being developed and adopted in WHO regions. The resulting rapid access to standardized data will increase the efficiency of programmatic response. It will also facilitate the work of regional laboratory managers or polio medical officers, who analyze and use data to motivate both field and laboratory staff to strive toward the performance goal of a sensitive and efficient virus surveillance system.
Good communication within the laboratory network and between the laboratory and the national and regional programs is critical to successful polio eradication. Frequent contacts between laboratory staff and program epidemiologists are required for establishing priorities for specimen testing, particularly when the laboratory anticipates testing or reporting delays. Participation of virologists on EPI committees for polio eradication and case classification at national and regional levels, frequent meetings with program staff, and informal personal contacts are all important channels of communication, with each serving different purposes.

**Certification of Polio Eradication**

The Americas, the first region to certify polio eradication, did so with a network of 9 laboratories using classical methods for virus detection and molecular methods for further characterization. Certification was based on the absence of virologically confirmed indigenous poliomyelitis cases over a 3-year period in the presence of adequate surveillance and the absence of detectable wild polioviruses from communities as determined by suitable environmental sampling methods or stool samples from selected high-risk populations or both [8].

Initially, clinical criteria were used for the classification of polio cases, the laboratory input being confined to the confirmation of a sometimes small percentage of cases, and the characterization and storage of endemic wild polioviruses for future use. Under this scheme, all AFP cases that resulted in residual paralysis as well as death or loss to follow-up were classified as polio cases.

Given the wide clinical spectrum of poliovirus infection and the occurrence of nonpolio residual paralysis indistinguishable from paralytic poliomyelitis, regions are now moving toward a case classification system that relies exclusively on the isolation of wild poliovirus for a diagnosis of poliomyelitis. The demand of certification commissioners for evidence that wild poliovirus has been sought in paralyzed children, healthy children, and the environment has implications for laboratory methodology and capacity.

Virologic surveillance will continue until certification that polio eradication has been achieved globally. The cost of testing the expected number of specimens over the next 8 years is estimated at US$50 million (excluding staff salaries and laboratory infrastructure costs, which are borne by national governments). This is ~3% of the cost of conducting the supplemental immunization needed to interrupt transmission of the virus. The calculated annual saving in vaccine costs will be US$1.5 billion upon cessation of immunization.

**The Future**

Questions remain to be answered on appropriate methods for supplemental poliovirus surveillance, including "environmental sampling" in countries without organized systems of feces disposal, methodology for sampling sewage and waste water, and parameters for performing stool surveys of healthy children.

There will be a continuing need to evaluate techniques that have the potential of increasing sensitivity, specificity, or efficiency of poliovirus detection. Genetically engineered mouse cell lines expressing the receptor for poliovirus promise great specificity. Direct detection of poliovirus by polymerase chain reaction may allow detection of specific wild viruses in very low concentration. New techniques will be introduced with due consideration to the advantages, feasibility, and ability to monitor performance.

Building a laboratory network is a dynamic process, with adjustments being made in light of experience and scientific advances. The goal is to provide a global laboratory resource that meets the current needs of polio eradication and will contribute to future control and elimination of other targeted diseases.

**References**