POLIO LAB NETWORK

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AFP Contact Stool Testing

The usefulness of testing stool specimens from acute flaccid paralysis (AFP) contacts has been the subject of considerable recent discussion.

The original recommendation to collect five stool samples from contacts of each AFP case was based on the need to increase the probability of detecting wild poliovirus circulation given that a proportion (>99%) of wild poliovirus infections are asymptomatic and AFP surveillance is not perfect.

The Global Commission on Polio Eradication, at its first meeting in February, 1995, also listed contact stools as one of the five criteria related to poliovirus surveillance. The Commission stated that: "For the previous three years, no wild virus will have been isolated from stool specimens collected from contacts of cases. Ideally, five contacts under 5 years of age living nearby will have been tested for each AFP case."

During the period immediately preceding the cessation of wild poliovirus circulation in the Americas, the testing of contact stools resulted in an increase case detection of 10%. This increase was achieved at an enormous cost to the laboratory network in terms of numbers of specimens processed and enteroviruses isolated. The problem of specimen load is only partially solved by pooling specimens, as discussed in the first 1995 issue of this Update. Concern has been expressed that the efficiency of the laboratory system is seriously reduced by the increased workload, resulting in tests on programmatically more important specimens being delayed or given less attention.

The WHO policy on contact stools is scheduled for discussion at a Global Technical Consultation planned for April 1996 in Geneva.

For the present, the WHO recommendation remains that stools should be collected from five contacts of each AFP case in countries with good surveillance systems. Where laboratories are being inundated by large numbers of specimens, a system of priority testing may be instituted at the discretion of the Polio Eradication Initiative (PEI) Manager. A suggested order of priority:

1. Case *and* contact stools from epidemiologically important AFP cases.
2. Stools from AFP cases (two from each).
3. Contact stools from polio compatible cases.
4. Contact stools from other AFP cases.

Implementation of a system of priorities will result in the important laboratory results reaching the PEI manager in the minimum time. Constant communication between virologists and epidemiologists is essential to achieve coordination and integration of all aspects of the poliovirus surveillance system.

In this issue:

REPORT FROM THE SOUTH EAST ASIAN REGION (SEAR)

Strengthening the Network

The Poliovirus Laboratory Network in SEAR currently consists of National Laboratories in Bangladesh, India (5), Indonesia (3), Sri Lanka, and Thailand. The laboratories in Delhi, Colombo, and Bangkok also serve as Regional Reference Laboratories. For the first three quarters of 1995, 1283 stools were processed with 296 being positive for poliovirus and 202 isolates sent to the three Reference Laboratories for intratypic differentiation. Wild poliovirus of all three types continue to circulate in the Region, primarily in India. A major goal of the Laboratory Network is to increase the number of stool specimens being processed in the Region and increase the number of poliovirus isolates submitted for intratypic differentiation.

Developing an Integrated Surveillance System

Considerable progress has been made in SEAR in developing and integrating a sensitive AFP surveillance system with a high quality laboratory network. However, some countries still require additional effort and resources to increase both field and laboratory capabilities. The Technical Consultative Group (TCG), in its December meeting, made a number of recommendations aimed at accelerating eradication in the Region. Recommendations relating to poliovirus surveillance include the following:

- Polio-endemic countries should develop specific plans of action to build an AFP surveillance system capable of monitoring eradication progress and providing guidance for immunization actions.

- Strengthening AFP surveillance will require a plan of action for training health workers, including clinicians, in the diagnosis and reporting of cases of AFP. The plan should also identify resource requirements and a strategy for obtaining necessary support.

- All countries should link the present laboratory data reporting system with case-based AFP surveillance data using a common epidemiologic identification number to provide timely and accurate information.

- Epidemiologic findings should be communicated across administrative borders to facilitate timely case investigation and outbreak response.

- For specimen testing, the highest laboratory priority should be assigned to specimens from AFP cases, followed by specimens from contacts of AFP cases which were virologically negative, but highly suspicious of being paralytic poliomyelitis.

- In low incidence countries, original stool specimens from cases clinically suspected as polio should be retained by the laboratory for retesting as needed.

- Proficiency test panels should be completed by each laboratory at least once per year.

- Voided stools should be the specimen of choice for viral culture. Specimens obtained by rectal tubes are a less preferable alternative. Rectal swabs are unacceptable.

- Date of latest OPV dose should be recorded and the information included with each specimen sent to the lab.

- All poliovirus isolates should be characterized as wild or vaccine-like. Wild poliovirus characterized by the Regional Reference Laboratories should be reported to the EPI manager by the submitting National Laboratory.

- Where necessary, active surveillance for AFP should be established with regular visits to key health facilities to conduct hospital record reviews and interviews of health providers, and to provide feedback to peripheral health units about the status of surveillance.

Recommendations on cell banking are discussed elsewhere in this issue.

Laboratory Report Forms are Revised

To facilitate linkage of laboratory results with individual AFP cases, all stool specimens are to be labeled with an epidemiologic identification number. Laboratory reporting forms are being revised to reflect AFP cases reported by stool culture results. Because some AFP cases may have more than one result, laboratory findings are to be reported in the following order of

priority: wild poliovirus, undifferentiated poliovirus, vaccine-related poliovirus, non-polio enterovirus, and no virus.

Contributed by: EPI/SEARO, New Delhi

Editorial note:

In 1996, SEAR, and the Laboratory Network, enter into a critical phase of the polio eradication initiative. This year, for the first time, all countries in the Region will be conducting NIDs or their equivalent. India vaccinated nearly 87 million children with OPV on 9 December 1995, perhaps the largest public health activity conducted on a single day in its history. All but one of the 32 States and Union Territories reported coverage of greater than 90% among children less than 3 years of age. On 20 January, India conducted its second Pulse Polio Immunization Day with early reports of success equal to or greater than the first.

With wild polio of all three types circulating in India and several neighboring countries, up until now it has been neither possible nor desirable to test stools from all AFP cases or subject all polio isolates to intratypic differentiation. India's phenomenal current success and Bangladesh's recent success with mass OPV immunizations renders that policy obsolete. SEAR is rapidly moving toward a virologic case definition and its laboratories can anticipate increasing expectations for high quality performance and timely reporting. The TCG recommendations reflect the critical need throughout the Region to improve and integrate field and laboratory operations to create a strong and responsive AFP surveillance system.

EVERY LAB NEEDS A CELL BANK

WHO recommends that every poliovirus laboratory maintain a liquid nitrogen bank of HEp2 and RD cells. Most labs do. A cell bank serves as insurance against the loss of working cells through contamination or other disasters. It also serves as a source of fresh cells for periodic replacement of working cells. Cells that have been passaged extensively, often under sub-optimal conditions, may have significantly reduced capacity for virus isolation.

The need for greater consistency in the practice of cell banking was discussed at meetings of the poliovirus laboratories of the Western Pacific Region in October and the South East Asian Region in November. Both Regions reached the same conclusions: a regular schedule for cell replacement should be introduced to standardize cell use and provide maximum sensitivity for isolating poliovirus and non-polio enteroviruses. The essence of the recommendations was as follows: *Each laboratory should maintain a bank of HEp2 and RD cells in liquid nitrogen. Cells should be of known pedigree obtained from a designated Regional Reference Laboratory. Cells used for virus isolation should be discarded after 20-25 passages or 6 months and replaced with cells with lower passage numbers from the cell bank.*

Key to successful cell banking is careful adherence to the WHO recommendations for storing cells in liquid nitrogen (-196°C) and reconstituting for use. Best results are achieved by freezing only healthy cells, freshly trypsinized and resuspended in 10% dimethyl sulfoxide medium at the recommended cell concentration of 4-8 $\times$ 10^6/ml. Cell suspensions are frozen slowly in a gaseous nitrogen apparatus in accord with instructions or in a partially insulated container placed overnight in a mechanical -70°C freezer. Best thawing results are achieved by immediately placing the frozen vials in a 36°C water bath. Survival and attachment of the newly thawed cells are improved by adding growth medium slowly, drop-by-drop, to reach the calculated minimum volume and transferring to fresh flasks to begin a new series of passages. For additional copies of the WHO recommended cell banking procedures and advice on banking practices, please fax or write Dr. Barbara Hull, Geneva, or contact the laboratory coordinator in your region.

ADDENDUM: The previous issue of UPDATE reported on page one that "The last confirmed wild poliovirus in China occurred in February 1994" and on page three that "Only one wild poliovirus isolate was reported from China in 1994." According to the final report of the Western Pacific regional Office (WPRO) for 1994, the total number of wild poliovirus isolates was six. The last wild isolate was associated with a case having onset of paralysis in September.

WESTERN PACIFIC REGION (WPR) LABS MEET

In October in Manila, representatives of all the WPR laboratories met for the first time. Until now, the major focus of the laboratory network has been directed towards the development and operation of effective surveillance systems in those countries where polio has been endemic. As eradication rapidly progresses in WPR, surveillance is being intensified and attention directed towards laboratory documentation requirements for certification of polio eradication. Documentation is required for all countries, those recently endemic as well as those free of polio for years.

Bringing together all the laboratories was critical for developing consensus on Region-wide issues and policies. Agreement was reached on the requirements for efficient and effective monitoring of stool specimens and isolates from collection to final results, the basic information needed for all AFP cases and contacts, a standard form for reporting results to WPRO, and a myriad of laboratory practices and procedures to assure high quality poliovirus isolation and characterization. Major conclusions emerging from the meeting were that:

- All poliovirus isolates, regardless of specimen origin, will be characterized as to wild type or vaccine related.
- Laboratory sensitivity, specificity, and timeliness will be continuously monitored to demonstrate adequacy of the poliovirus surveillance system.
- All National Laboratories will provide a monthly report of results to WPRO according to a standard format. The information will be used to update country summaries, monitor laboratory performance, and coordinate international agency activity.

YOU MAY BE ASKED. . .

Why not a rectal swab?

Clinicians, nurses, and field investigators are busy people. Often they don't have time to wait for a stool specimen or return for one, particularly if travel is difficult. Collection of the second specimen presents an even greater challenge. For many, the rectal swab would seem to be the answer. Why not?

The *1995 WHO Field Guide for Supplementary Activities Aimed at Achieving Polio Eradication*, which all EPI and laboratory managers should have on their desks, specifies that a stool specimen be collected within 14 days of onset of paralysis and a second stool specimen be collected 24-48 hours later (the one exception for the requirement for a second stool is in the Americas where eradication has already been declared). Page 70 of the Field Guide describes the proper procedure for collecting a stool specimen and lists the rectal tube as an alternative but less preferred method. The rectal swab is not mentioned.

Experience has shown the rectal swab is not a satisfactory procedure. First, the specimen collected by rectal swab may be hundreds-fold less than the recommended 8 grams of stool, thus reducing the likelihood of virus isolation and virtually precluding storage and repeat testing. Second, the rectal swab may dry, further decreasing virus survival. Third, many rectal swabs in reality are anal swabs and contain no fecal material at all.

The rectal swab initially may appear to be the most efficient procedure, but, in the long run, it may be the least. All of the effort required for collecting, shipping, testing, reporting, and recording may be wasted on a specimen that was unlikely to have contained a virus anyway. Even worse, a wild type poliovirus may have been missed.

Good poliovirus surveillance begins with a good stool specimen, and the rectal swab isn't it.

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