TRANSPORT OF MICROBIOLOGICAL SPECIMENS TO A TROPICAL COMMUNICABLE DISEASE CENTER[1, 2]

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Practical and efficient transport of clinical specimens is an essential part of public health laboratory and epidemiologic work. This article describes a specimen transport system devised for Bahia, Brazil, whose features should be applicable in some degree to many other tropical developing areas.

Introduction

In 1977 the State of Bahia, Brazil, completed renovation of the State Public Health Central Laboratory in connection with a Federal Ministry of Health program designed to create 17 new laboratories in health posts throughout the state. The central laboratory was to provide auxiliary diagnostic support for the small local laboratories. The state epidemiologic service also required central diagnostic facilities to deal with specimens from the interior of the state.

In connection with this program, it was necessary to devise a system for transporting specimens that would keep pathogenic microorganisms viable for two or more days without refrigeration at temperatures of up to 30°C. No standard system for transporting specimens was available. Systems used elsewhere, such as those in the United States (*1, 2*), provided potentially useful models; but these models needed modification to allow for long-distance transport, usually by public bus, from laboratories lacking facilities for preparing or storing complex materials at tropical temperatures. A transport system for microbiological specimens was therefore devised. This article describes the system—appropriate for transport of sera, feces, sputum, swabs of lesions, skin scrapings, smears, and other clinical materials for viral, bacterial, fungal, and parasitic studies—that was developed and adopted for use in the State of Bahia.

Specimen Transport Techniques

The first step in developing a transport system was to prepare detailed information for the interior laboratories concerning collection and inoculation of clinical specimens to be transported. The laboratories were instructed about preparing material for transport that would be subjected to microscopy, culture, and serologic testing.

The recommendations of the United States Center for Disease Control (*1*) and the American Society for Microbiology (*2*), as modified for this purpose, are summarized in Table 1. The types of specimens needed, the required materials, and the maximum viability periods are listed. The major disease-causing bacteria, viruses, and fungi remain viable in the indicated transport media for at least two days. Viral, mycobacterial, fungal, and serum specimens are best transported in an ice chest. However, only specimens that might contain *Histoplasma* or *Actinomyces* require refrigeration during transport. Other spe-

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Table 1. Specific transport materials and methods.

Estimated cost of consumable materials per 100 specimens[a]	Examination for:	Specimen	Materials	Maximum viability period (days)
U.S.$5.00	*C. diphtheriae, Streptococcus, Staphylococcus*	Throat or lesion swab	Silica gel, 10 x 75 mm glass tube, stopper,[b] swab	3
U.S.$10.00	*Salmonella, Shigella, Yersinia,*	Stool swab	Gary-Blair transport medium (5 ml in 10 x 75 mm tube), stopper,[b] swab	2
U.S.$12.00	Poliovirus	(a) Stool swab	5 ml of charcoal viral transport medium, 10 x 75 mm tube, stopper,[b] swab	21
		(b) 5-10 g stool	Watertight container, ice	2[d]
U.S.$1.00	Serum antibodies	3-5 ml of serum	10% sodium azide solution, 10 x 75 mm tube,[c] stopper[b]	3
U.S.$5.00	Conditions detectable by microscopy	Smear of material	Glass slide, wooden stick	5
U.S.$25.00	Mycobacteria	Sputum or other material	Cetylpyridinium chloride-NaCl solution and plastic containers (screw-top); or ice for the containers if CPC solution is not used	8 1[d]
U.S.$5.00	Dermatophytes	Hair or skin	Two paper envelopes	7
U.S.$25.00	Systemic fungi (except *Histoplasma*)	Appropriate material	Two Sabouraud's slants in 10 x 75 mm tubes, one with antibiotics	2
U.S.$25.00	*Histoplasma, Actinomyces*	Appropriate material	Saline-antibiotic solution, a 25-50 ml plastic cup with top, ice	2[d]
U.S.$15.00	Intestinal parasites	Stool part stool, 3 parts	5 ml of polyvinyl alcohol fixative, 5 ml of 10% formalin, 2 small 10 ml glass vials with tops	60

[a]Based on dollar values January 1979.
[b]Using a rubber stopper and sealing the tube with tape is recommended. Tubes sealed with screw-caps and ordinary corks are apt to leak.
[c]The tubes should be sterile to decrease contamination.
[d]If the specimen is chilled in an ice chest.

cimens do not need refrigeration unless temperatures exceed 30°C.

Necessary consumable materials include Cary-Blair media (BBL, Beckon, Dickinson & Co., P.O. Box 243, Cockeysville, Maryland, U.S.A.), charcoal viral transport and Sabouraud's media (BBL), sodium azide, polyvinyl alcohol-fixative,[4] 10 per cent formalin, cetylpyridinium chloride-NaCl (Sigma Chemical Company, St. Louis, Missouri, U.S.A.), antibiotic solutions, swabs, envelopes, plastic screw-cap containers, wooden sticks, glass slides, and ice. The estimated cost of these consumable materials per 100 specimens is listed in Table 1.

Reusable materials include silica gel (Merck, West Germany), 10 x 75 mm glass tubes, 10-ml glass vials, rubber stoppers for 10 x 75 mm tubes, and plastic watertight mailing containers. The following text subsections provide specific details on transport of different types of specimens.

[4](PVA powder and PVA fixative solution, Delkote, Inc., Wilmington, Delaware, U.S.A.).

C. diptheriae, *Streptococci, and Staphylococci*

Lesions suspected of containing these organisms are rubbed with a sterile swab. Calcium alginate or Dacron swabs offer some advantage, in that they are less absorbent than cotton and therefore yield higher bacterial counts after plating. The swab is placed in a small glass tube with 3-5 g of desiccated silica gel, and the tube is tightly sealed with a stopper and tape (*3, 5, 6*). The organisms will remain viable for at least three days at ambient temperatures. The swabs are later moistened in a few ml of broth before plating onto media, a step that helps to remove bacteria from the swab (*3, 11, 18, 19*). The tubes, rubber stoppers, and silica gel are washed and reused. The silica gel is sterilized and redesiccated by heating for several hours in a dry oven at 170°C.

Enteric Bacterial Pathogens

Stool specimens are collected on cotton-tipped swabs and stab-inoculated into 5 ml or Cary-Blair transport medium (*1, 2*) in glass tubes. The swab stick, which is broken, remains in the medium, and the tube is then closed tightly with a stopper, taped, and labeled. This will maintain the viability of *Salmonella, Shigella, Yersinia,* and *Vibrio* microorganisms for at least two days at ambient temperatures.

Although Cary-Blair medium is available commercially, it may be prepared easily and inexpensively as follows: mix sodium thioglycolate (1.5 g), disodium phosphate (1.1 g), sodium chloride (5.0 g), agar (5.0 g), and warm distilled water (991.0 ml). After cooling to 50°C, add 9.0 ml of fresh 1 per cent calcium chloride, and adjust the pH to 8.4 with 1N NaOH or 1N HCl. Distribute the media into 10 x 75 ml tubes, steam for 15 minutes, and stopper tightly (*1*). These media-filled tubes may be stored at a temperature range of 5-25°C for up to six months.

Poliovirus

Stool specimens are inoculated into the charcoal viral transport medium (CVTM) developed by Leibovitz (*4*). A swab of fecal matter is pressed into 5 ml of media contained in a 10 x 75 mm glass tube, and the tube closed tightly with a rubber stopper and tape. Viruses remain viable at high titers for at least 21 days when kept at temperatures of up to 25°C.

This CVTM is prepared by mixing sodium chloride (4 g), potassium chloride (0.2 g), dipotassium phosphate (1.7 g), charcoal (10 g of an activated-neutralized product obtained from the Sigma Chemical Co., St. Louis, Missouri), agar (4 g), and distilled water (1,000 ml). The pH is adjusted to 7.6; the media is then distributed into 10 x 75 mm tubes and autoclaved. An alternative method is to transport the stool specimen (a 5-10 g specimen) in a tightly sealed waterproof container that is chilled on ice or (preferably) frozen.

Serum

Serum for antibody testing is collected as aseptically as possible in small sterile glass tubes that are sealed with a stopper and tape. The stoppers should be autoclaved or rinsed in 70 per cent alcohol before use. One drop of a stock solution of 10 per cent sodium azide is added per 5 ml of serum (final concentration, 0.1 per cent) to retard bacterial growth. As an alternative, one drop of 1 per cent Merthiolate can be added for each 5 ml of serum (*1*). Maximum care should be taken to prevent contamination, which interferes with complement fixation tests and (depending upon the contaminating organism) may interfere with other serologic tests as well. Chilling of the specimens retards growth of bacterial contaminants (*1, 2*). The antibody titers of sera stored at

5°C for several months do not change significantly.

Microscopy Specimens

Smears for gram, acid-fast, mycology, or blood-protozoan staining are prepared on slides from the most mucopurulent or sanguinous part of the specimen. They must be thoroughly air-dried and then taped between two wooden sticks (to prevent breakage) for transport (*1, 2, 9*). Tongue depressors are convenient for this purpose. Bacterial and fungal contamination may occur in humid areas if the slides are kept for a long time before being stained and examined.

Micobacteria

Sputum for culture is mixed with an equal volume of a cetylpyridinium chloride (1 per cent)-sodium chloride (2 per cent) solution (CPC-NaCl) in small plastic screw-cap cups. This solution digests the thick sputum and inhibits the growth of other bacteria during transport. Mycobacteria remain viable in this solution for up to eight days (*8*). Specimens can be sent in well-sealed plastic containers on ice without CPC-NaCl solution if the transport time does not exceed 24 hours (*1, 2, 7*).

Mycological Specimens

Dermatophyte specimens usually consist of skin or hair scrapings. These specimens are placed in a paper envelope—which in turn is folded, labeled, sealed, and then placed inside a second envelope that can be mailed to the laboratory. Specimens remain viable for up to seven days at temperatures of up to 30°C if they are kept dry.

Systemic fungal specimens should be inoculated on each of two Sabourand agar slants, one without antibiotics and one containing 100 μg/ml of chloramphenicol and 0.5 mg/ml of cycloheximide (Sigma Chemical Co.—*2*). These tubes, after being stoppered tightly and taped, can be sent to the laboratory at temperatures ranging up to 30°C. Viability is assured for up to three days.

Specimens suspected of containing *Histoplasma* or *Actinomyces* should be placed in a small amount of saline with chloramphenicol (0.05 mg/ml) and should be kept on ice while being transported to the laboratory. The transport time must not exceed 48 hours.

Sabouraud's media is prepared by mixing dextrose (40 g), peptone (10 g), agar (15-20 g), and water (1,000 ml). The pH is adjusted to 5.6, and 5 ml portions of media are distributed into 10 x 75 ml tubes and autoclaved. If kept tightly sealed it may be stored up to six months at 5°C.

Protozoan Cysts and Trophozoites, and Helminth Eggs and Larvae

These specimens are best preserved by the two-vial (polyvinyl alcohol fixative and 10 per cent formalin) technique (*1,2*). The PVA fixative consists of 5 per cent glacial acetic acid, 1.5 per cent glycerol, and 5 per cent PVA in Schaudinn's fixative (one part 95 per cent ethyl alcohol and two parts saturated aqueous mercuric chloride solution). It is very effective for preserving cysts and trophozoites if fresh stool is rapidly and thoroughly mixed with the solution (*2,20*). The 10 per cent formalin in the second vial preserves helminth eggs, helminth larvae, and protozoan cysts. Both vials should be closed tightly and taped.

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All specimens, whatever their nature, should bear a carefully typed or penciled label (ink pens and wax pencils smear) giving the date and time of collection. A brief clinical history and the etiologic agent suspected should be indicated on a form accompanying the specimen. Care should be

taken to eliminate tubes or vials with cracks or chips, and all vessels should be carefully sealed and packed to prevent breakage. All fluid specimens should be packed in absorbent cotton or paper towels inside a plastic or Styrofoam leak-proof container. The laboratory address and the sender's return address should be clearly printed on the outside container label. A label saying "Fragile-Biologic Medical Materials" is attached to warn handlers in case of leakage or breakage of specimens.

Discussion

This transport system satisfies the basic requirements for transport of microbiological specimens in a tropical environment. The procedures specified provide for maximum viability while limiting themselves to inexpensive and simple materials that can easily be obtained, prepared, and stored.

Silica-gel techniques are as reliable as direct culture for *C. diphtheriae* (*3*) and streptococci (*5,6*). They are effectively utilized in a "mail-in" method employing aluminum foil packets. Alternative methods for transporting streptococci and *C. diphtheriae* under field conditions include procedures using selective media (*3,12,13*), especially prepared swabs (*14,15*), transport media, and filter-paper strips (*16,17*). Although the selective media method was found effective under tropical conditions, the silica-gel swab technique is the method of choice because bacterial survival rates are similar and the problems of transporting the media and inoculating it in the field are eliminated (*5*). Other transport media (Amie's, Stuart's, and Cary-Blair media) are unsatisfactory because of poor recovery rates. These result primarily from overgrowth by other rapidly growing organisms also present in the inoculum.

Cary-Blair transport medium is the most widely recommended enteric bacterial transport medium (*1,2*). Alkaline-peptone water, Amie's, and Stuart's transport media are also available. *Salmonella, Shigella, Yersinia,* and *Vibrio* species usually remain viable in the Cary-Blair medium for at least two days. It is available commercially, or is easily prepared, and is quite stable during storage.

Charcoal viral transport medium (CVTM) is a simple and reliable transport medium for enteroviruses. Leibovitz has demonstrated that CVTM is superior to Dextran-viral transport medium and to Amie's medium, and that it provides results comparable to those attained with throat washings and direct bedside inoculation of tissue cultures (*4*). Influenza viruses and adenoviruses have also been recovered in high titers after 14 and 21 days at temperatures of up to 25°C. Other than freezing (preferably at -70°C), few alternative techniques are offered for long-distance or long-term preservation of virologic specimens. The recommended procedure is to inoculate the appropriate host material as soon as possible without the use of preservatives or fixatives.

Mycobacterial specimens may become overgrown with other microorganisms after 24 hours, even when chilled. The CPC-NaCl decontamination-preservation technique provides an inexpensive and effective way of transporting specimens without refrigeration. The material employed digests the thick sputum and decontaminates the specimen during the transit period. Ambient temperatures over 30°C rapidly increase the rate of the digestion-decontamination process, however, and reduce the viability period. The mycobacterial isolation rates obtained after eight days of this treatment compared favorably with those obtained by using the n-acetyl-l-cysteine and sodium hydroxide methods, and the atypical mycobacteria isolation rate was actually higher with the CPC-NaCl technique (*8*). As this latter process requires a minimum of 24 hours, however, specimens that can be processed in the laboratory on

the day of collection should be transported without this solution in a container of ice.

Dermatophyte specimens may be sent through the mail in envelopes. They may also be inoculated directly onto two Sabouraud's media slants, one with antibiotics and the other without. Direct inoculation may result in a slightly higher isolation rate.

Chromoblastomycosis, mycetoma, and sporotrichosis specimens (containing granules, pus, serosanguinous fluid, etc.) are best inoculated directly onto this medium prior to transport. The systemic mycoses can also be directly inoculated onto Sabouraud's media slants in tubes.

Specimens suspected of containing *Histoplasma* or *Actinomyces* are more difficult to handle, since these organisms are more fastidious. They may be inoculated onto the two Sabouraud's slants; better recovery can be expected, however, if a large portion of the fresh clinical material is placed in an antibiotic solution on ice and sent to the laboratory within 48 hours. Other media recommended for these latter organisms (*1,2*) are less practical for use in the field. Antibiotic solutions decrease the growth of bacterial flora and thus tend to increase the fungal isolation rates (*1,2,9*).

The transport methods described herein maintain the viability of pathogenic microorganisms for at least two days. This time period permits specimens to be transported by motor vehicles, including public buses. The geographic area for which these transport methods have been designed is the State of Bahia. Ninety per cent of the state's population lives within one day's bus travel time from the central laboratory in Salvador, making this a practical means of retrieving specimens from the field.

SUMMARY

Practical and efficient means of transporting clinical specimens in the tropics are essential to any program of public health laboratory and epidemiologic work. This article outlines a system for transport of microbiological specimens that was designed for use in the State of Bahia, Brazil. The system's essential features include use of inexpensive materials with long storage potentials, employment of simple techniques, and identification of methods that keep microorganisms viable for at least 48 hours during transport.

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WORLD HUNGER*

The August-September 1979 issue of *World Health* is a double-issue devoted entirely to nutrition. The approach of making people dependent on food hand-outs from wealthier countries has proved to be no answer to world hunger. Now, people are being taught how to improve their own nutrition by producing their own food, e.g., the cultivation of fish among the rice of paddies, the value of land reform programs aimed at helping small farmers and the landless, and the replacement of local goats by much bigger breeds giving four times as much milk.

The issue also questions the effects of food additives that increase food supply and improve nutrition; defines the roles of carbohydrates and fats in our diet; and discusses health workers specializing in nutrition, and the importance and decline of breastfeeding.

*WHO press release, 18 September 1979.