

Chapter 2

Collection and Transport of Fecal Specimens

Fecal specimens should be collected in the early stages of any enteric illness, when pathogens are usually present in the stool in highest numbers, and before antibiotic therapy has been started (Table 2-1).

Table 2-1. Collection and transport of specimens for laboratory diagnosis

When to collect	When the patient is having diarrhea, as soon after onset of illness as possible (preferably within 4 days of onset) and before antimicrobial treatment is started.
How much to collect	Rectal swab or swab of fresh stool in transport medium.
Transport medium	Cary-Blair or other suitable transport medium (NOT buffered glycerol saline for <i>V. cholerae</i>).
Storage after collection	Refrigerate at 4°C if the specimens will be received by the laboratory within 48 hours, or freeze at -70°C. Fecal specimens from patients with suspected cholera can be transported at ambient temperature and held for longer times if necessary; however, refrigeration is preferred.
Transportation	Seal tubes/containers to prevent leakage; place in waterproof container to protect from wet or dry ice. Ship in insulated box with ice packs, wet ice, or dry ice by overnight delivery.

Stool specimens or rectal swabs should be collected from 10-20 persons who meet the following criteria:

- Currently have watery diarrhea (cholera) or bloody diarrhea (dysentery)
- Had onset of illness less than 4 days before sampling
- Have not received antimicrobial treatment for the diarrheal illness

A. Collection of Stool

Collect stools from patients in clean containers without disinfectant or detergent residue and with tight-fitting, leak-proof lids. Specimens should not be collected from bedpans, as they may contain residual disinfectant or other contaminants. Unpreserved stool should be refrigerated if possible and processed within a maximum of 2 hours after collection. Specimens that cannot be cultured within 2 hours of collection should be placed in transport medium and refrigerated immediately.

1. Placing stool in transport medium

A small amount of stool can be collected by inserting a sterile cotton- or polyester-tipped swab into the stool and rotating it. If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab. Immediately insert the swab into transport medium. (The transport medium should have been chilled for 1 to 2 hours, if possible.) The swab should be pushed completely to the bottom of the tube of transport medium and the top portion of the stick touching the fingers should be broken off and discarded. Replace the screw cap and tighten firmly. Place the tube in a refrigerator or cold box.

2. Collection of rectal swabs

Rectal swabs may be collected as follows: moisten the swab in sterile transport medium, insert through the rectal sphincter 2 to 3 cm (1 to 1.5 inches) and rotate, withdraw and examine to make sure there is some fecal material visible on the swab. Immediately insert the swab into cold transport medium as described in above paragraph. Place the tube in a refrigerator or cold box.

The number of swabs needed will depend on the number of plates to be inoculated. In general, if specimens will be examined for more than one pathogen, at least two stool swabs or rectal swabs should be collected per patient, and both swabs should be inserted into the same tube of transport medium.



Figure 2-1. Cary-Blair semisolid transport medium

3. Transport media

Cary-Blair transport medium

Cary-Blair transport medium can be used to transport many enteric pathogens, including *Shigella*, *Vibrio cholerae*, and *Escherichia coli* O157:H7 (Figure 2-1). Cary-Blair's semisolid consistency provides for ease of transport, and the prepared medium can be stored after preparation at room temperature for up to 1 year. Because of its high pH (8.4), it is the medium of choice for transport and preservation of *V. cholerae*.

Preparation and quality control of Cary-Blair

Prepare according to manufacturer's instructions. [Note: There are several commercially available dehydrated formulations of Cary-Blair. Some require the addition of calcium chloride and some do not. Cary-Blair can also be prepared from individual ingredients.] When Cary-Blair is prepared, it should be dispensed into containers in sufficient volume so that swabs will be covered by at least 4 cm of medium. For example, 5- to 6-ml amounts may be dispensed into 13 x 100-mm screw cap tubes. With the caps loosened, sterilize by steaming (do not autoclave) at 100°C for 15 minutes. Tighten the caps after sterilization. Cary-Blair is quite stable if stored in tightly sealed containers in a cool dark place so that the medium does not dry out. Cary-Blair may be used for up to 1 year as long as there is no loss of volume, contamination, or color change.

Other transport media

Other transport media that are similar to Cary-Blair are Amies' and Stuart's transport media. Both of these are acceptable for *Shigella* and *E. coli* O157:H7, but they are inferior to Cary-Blair for transport of *V. cholerae*.

Alkaline peptone water (APW) may be used to transport *V. cholerae*, but this medium is inferior to Cary-Blair and should be used only when the latter medium is not available. APW should not be used if subculture will be delayed more than 6 hours from the time of collection because other organisms will overgrow vibrios after 6 hours.

Buffered glycerol saline (BGS), a transport medium that is used for *Shigella*, is unsuitable for transport of *V. cholerae*. Additional disadvantages of BGS are that it can be used for only 1 month after it is made and, being a liquid medium, is more likely to leak or spill during transport.

4. Storage of specimens in transport medium

If transport medium has been stored at room temperature, it should be chilled, if possible, for 1 to 2 hours before use. Specimens preserved in transport medium should be refrigerated until processed. If specimens will be kept more than 2 to 3 days before being cultured, it is preferable to freeze them immediately at -70°C. It may be possible to recover pathogens from refrigerated specimens up to 7 days after collection; however, the yield decreases after the first 1 or 2 days. Prompt

plating, refrigeration, or freezing of specimens in Cary-Blair is particularly important for isolation of *Shigella*, which is more fragile than other enteric organisms. Fecal specimens in transport medium collected from patients with cholera need not be refrigerated unless they are likely to be exposed to elevated temperatures (>40°C).

5. Unpreserved specimens

When transport medium is not available, one option for suspect *V. cholerae* specimens is to soak a piece of filter paper, gauze, or cotton in liquid stool and place it into a plastic bag. The bag must be tightly sealed so that the specimen will remain moist and not dry out. Adding several drops of sterile saline to the bag may help prevent drying of the specimen. Refrigeration during transport is desirable but not necessary. This method is not suitable for transport of *Shigella* or *E. coli* O157:H7 specimens and is less effective than transport medium for preserving *V. cholerae* organisms.

B. Preparing Specimens for Shipment

Specimen tubes should be clearly labeled with the specimen number, and if possible, the patient's name and date of collection. Write the numbers on the frosted portion of the specimen tube, using an indelible marker pen. If there is no frosted area, write the information on a piece of first-aid tape and fix this firmly on the specimen container. Patient information should be recorded on a data sheet; one copy should be sent with the specimens and another kept by the sender. A sample data sheet is provided in Annex F.

If a package is to be shipped by air, refer to packaging regulations presented in the publication, *Dangerous Goods Regulations (DGR)*. *International Air Transport Association (IATA)*. These regulations are summarized in Chapter 13, "Packing and Shipping of Clinical Specimens and Etiologic Agents." Even if the package will be shipped by other means, these regulations are excellent guidelines for packing all infectious or potentially infectious materials.

1. Refrigerated specimens

Refrigerated specimens should be transported to the laboratory in an insulated box with frozen refrigerant packs or ice. If wet ice is used, place the tubes or containers in waterproof containers such as plastic bags that can be tightly sealed to protect the specimens from the water formed by melting ice.

2. Frozen specimens

Frozen specimens should be transported on dry ice. The following precautions should be observed:

- Place tubes in containers or wrap them in paper to protect them from dry ice. Direct contact with dry ice can crack glass tubes.
- If the specimens are not in leakproof containers, protect them from exposure

to carbon dioxide by sealing the screwcaps with tape or plastic film or by sealing the tubes in a plastic bag. Carbon dioxide will lower the pH of the transport medium and adversely affect the survival of organisms in the specimen.

- Ensure that the cool box is at least one-third full of dry ice. If the specimens are sent by air and more than 2 kg of dry ice is used, special arrangements may be necessary with the airlines. Airlines accept packages with less than 2 kg of dry ice.
- Address the package clearly, including the name and telephone number of the receiving laboratory. Write in large letters: EMERGENCY MEDICAL SPECIMENS; CALL ADDRESSEE ON ARRIVAL; HOLD REFRIGERATED (or “FROZEN” if applicable). Be sure that all applicable labels and forms, such as those required by IATA, are correctly fixed to the outside of the package.

References

Centers for Disease Control and Prevention. Recommendations for the collection of laboratory specimens associated with outbreaks of gastroenteritis. MMWR 1990;39 (No. RR-14).

Centers for Disease Control and Prevention. Laboratory methods for the diagnosis of *Vibrio cholerae*. Atlanta, Georgia: CDC, 1994.

