

III. Collection and Transport of Patient Specimens

Inadequately or improperly collected or transported specimens can hamper the efforts of the laboratory to recover *Vibrio cholerae* O1. Therefore, it is crucial to any laboratory investigation to collect suitable specimens, use an appropriate transport medium for fecal specimens, and transport specimens to the laboratory in a timely manner (see Table III-1).

A. Collection of Specimens

1. 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.

Collection of stool

Collect stools from patients in clean (no disinfectant or detergent residue) containers with tight-fitting, leak-proof lids. Specimens should not be collected from bedpans, as they may contain residual disinfectant. A rectal catheter may be used to collect liquid stool from suspected cholera patients. A sterile catheter is lubricated with sterile liquid, inserted past the rectal sphincter, and gently moved into the intestine until liquid stool begins to flow from the end of the catheter.

Unpreserved stool should be refrigerated if possible and processed within a maximum of 2 hours after collection. Any drying of the specimen or a shift to acid pH will significantly reduce the numbers of viable organisms. If the specimen will be kept for longer than 2 hours before processing, a sterile cotton- or polyester-tipped swab should be immersed into the stool and placed in Cary-Blair transport medium (see Section B of this chapter for instructions on inoculation of Cary-Blair). If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab. Specimens preserved in transport medium need not be refrigerated unless they are likely to be exposed to elevated temperatures (>40°C).

Collection of rectal swabs

Moisten a sterile cotton-tipped swab with Cary-Blair transport medium (lubricants must not be used), insert the swab through the rectal sphincter into the anal canal, and rotate several times. Examine the swab for evidence of fecal material. Place the swab into the tube of Cary-Blair medium and transport to the laboratory at room temperature or refrigerated (4°C).

Table III-1. Collection of specimens for laboratory diagnosis of cholera

Collection instructions	Fecal specimens	Serum specimens
When to collect	During period of active diarrhea (as soon after onset of illness as possible)	Acute-phase serum within 3 days after onset if possible; convalescent-phase serum 10-21 days after onset ^a
How much to collect	Rectal swab or swab of fresh stool	10-15 ml whole blood from adults or 3-5 ml from children
Method of collection	Stool or rectal swab placed in Cary-Blair transport medium	Collect blood in tubes without anticoagulants; clot and centrifuge; place serum in sterile tube
Storage of specimen after collection	Store at room temperature or refrigerate at 4°C	Immediately refrigerate or freeze serum (do not freeze whole blood)
Transportation	Seal samples to prevent leakage; ship in durable container by overnight delivery	Place sealed samples on ice packs or dry ice; ship in insulated box by overnight delivery

^a Vibriocidal antibody titers begin to rise several days after onset and peak by 10-21 days.

2. Serum for antibody studies

Obtain two serum specimens (an acute-phase specimen and a convalescent-phase specimen). Obtain the acute-phase serum specimen as close to the time of onset of illness as possible (ideally within 0 to 3 days after onset of illness) and the convalescent-phase serum specimen 10 to 21 days after the onset of illness. Indicate the date the blood specimen was drawn and the date of onset of disease in addition to the name and age of the patient.

Collect blood specimens from adults (10 to 15 ml) and from children (3 to 5 ml) in tubes that do not contain anticoagulants. Allow blood to clot. Centrifuge the blood and send only the serum for analysis. If no centrifuge is available, store the blood specimens in a refrigerator until a clot has formed; then carefully remove the serum with a Pasteur pipette, avoiding red blood cells. Place the cell-free serum into a sterile screw-capped tube. If the serum is to be held for several days, freezing is preferable to refrigeration to prevent bacterial growth. If possible, ship frozen specimens on dry ice. However, if serum contains red blood cells, it should not be frozen because this will cause lysis of the cells and make analysis difficult. If the serum has been drawn off without centrifuging using the clotting technique described above, ship the specimens refrigerated (not frozen), so that they can be centrifuged to remove any remaining red blood cells before freezing.

B. Transport Media

1. Cary-Blair

Cary-Blair transport medium is the medium of choice for the preservation and transport of *V. cholerae* because its high pH (8.4) is optimal for this species and its semisolid consistency provides for ease of transport. Cary-Blair medium is stable and can be stored after preparation for up to 1 year in tightly sealed containers. It can be used to transport many enteric pathogens besides *V. cholerae*. (See Chapter XI, "Preparation of Media and Reagents" for instructions on how to prepare Cary-Blair medium.)

Inoculation of Cary-Blair transport medium

After collecting the specimen, aseptically insert one or two swabs into the tubed medium (Figure III-1). Keep the head of the swab submerged in the medium. Aseptically break off the end portion of the swab stick that has been touched by the fingers. Replace the screw cap tightly. Transport or store inoculated medium at room temperature (25°C) or refrigerated (4°C).

2. Other transport media

Alkaline peptone water (APW) may be used to transport *V. cholerae* for short periods if Cary-Blair is not available. APW should not be used if



Figure III-1. Cary-Blair semisolid transport medium

subculture will be delayed more than 6 hours from the time of collection because other organisms will overgrow vibrios after 6 hours.

Amies' (pH 7.4) or Stuart's (pH 7.3) transport media may be used to transport specimens if Cary-Blair is not available but only for short periods (1-2 days) because the pH of these media is not optimal for *V. cholerae*. Buffered glycerol saline is not suitable for transport of *V. cholerae* and must not be used.

C. Unpreserved Specimens

If transport media cannot be used, one option 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.

D. Transport of Specimens

The transport or shipment of diagnostic specimens by public or commercial delivery systems may be subject to local or national regulations. Transport of specimens should not present a hazard to humans or the environment and should protect the viability of suspected pathogens. For instructions about the international shipment of diagnostic specimens and etiologic agents, see Chapter XII, "Storage and Shipment of Isolates."

The following guidelines are suggested for the transport of diagnostic specimens in the absence of specific regulations:

- Place each specimen in an appropriate primary container (test tube, stool cup, transport medium, serum vial).
- Place primary containers in a durable covered waterproof secondary container with sufficient blotting material (e.g., cotton wool, paper towels) to absorb any leakage from the primary container.
- Specimens may be refrigerated by placing them in an insulated box with frozen refrigerant packs (these may be commercial or homemade). If specimens are refrigerated with wet ice instead of refrigerant packs, water from the melting ice should not seep into the specimen tubes or leak from the secondary container. If wet ice is used, place the specimen containers in waterproof plastic bags that can be tightly sealed.
- Frozen specimens can be kept frozen only by shipping them on dry ice (solid CO₂). Use enough dry ice to keep the specimen frozen until it is received at the laboratory that will process it (usually about one-third to one-half of the shipping container). Glass tubes should not come into direct contact with dry ice; cushion specimens with a layer of packing paper or other buffering material between the tubes and the dry ice.
- Pack specimens on dry or wet ice securely, so that they will not roll loosely in the container after the ice has dissipated.
- Specimens that are not refrigerated or frozen should still be protected against possible extremes of heat or cold.
- Select the most rapid and reliable means of transportation to avoid delays in shipment. If air transport is used for frozen specimens, obtain prior approval from the airline authorizing the use of dry ice, because CO₂ gas is released as it melts. Schedule delivery during business hours on a weekday if possible.
- If specimens are shipped by mail, pack samples according to national and/or international postal requirements (see Chapter XII.) Be sure to attach all necessary information and documentation for each specimen.

References

1. World Health Organization. Manual for the Laboratory Investigations of Acute Enteric Infections. Geneva: World Health Organization, 1987; publication no. WHO/CDD/83.3 rev 1.
2. CDC. Recommendations for the collection of laboratory specimens associated with outbreaks of gastroenteritis. MMWR 1990;39 (No. RR-14).
3. Cary SG, Blair EB. New transport medium for shipment of clinical specimens. I. Fecal specimens. J Bacteriol 1964; 88:96-8.
4. Global Task Force on Cholera Control. Guidelines for cholera control. Geneva: World Health Organization, 1992; publication no. WHO/CDD/SER/80.4 rev 4.