

XII. Storage and Shipment of Isolates

A. Storage of Isolates

Pure growth of *Vibrio cholerae* will usually remain viable for several days on solid medium held at room temperature (22° to 25°C) unless the medium dries out or becomes acidic. However, if cultures are to be maintained for longer than a few days, they should be appropriately prepared for storage (Figure XII-1). Selection of a storage method depends on the length of time the organisms are to be held and the laboratory equipment and facilities available.

1. Short-term storage

Vibrios should never be stored on a carbohydrate-containing medium, because acidic byproducts of metabolism quickly reduce viability. Blood agar base (BAB), Trypticase soy agar (TSA), T₁N₁, and heart infusion agar (HIA) are good storage media for vibrios. Nutrient agar should not be used since it has no added salt and *V. cholerae* does not grow as well as on a NaCl-contaminated medium such as BAB, TSA, or HIA. Prepare storage medium and dispense in 3- to 4-ml amounts in small tubes (approximately 13 x 100 mm) and sterilize at 121°C for 15 minutes. While the tubes are still hot, place them in a slanted position to provide a short slant and deep butt.

To inoculate, stab the inoculating needle to the butt of the medium once or twice, then streak the slant. Incubate overnight at 35° to 37°C.

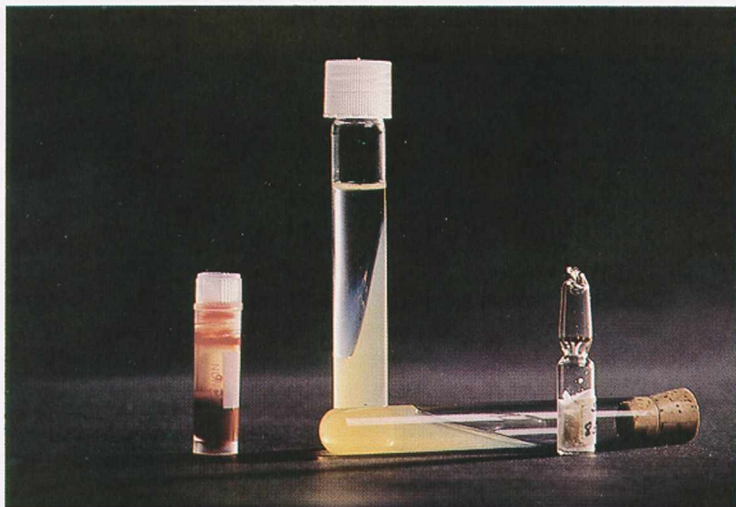


Figure XII-1. Various methods of storage of isolates (left to right): a cryovial, stock slant with mineral oil, agar deep with paraffin cork, and a lyophil.

Seal the tube with cork stoppers that have been soaked in hot paraffin or treated in some other way to provide a tight seal. Store cultures at room temperature (about 22°C) in the dark. Sterile mineral oil may also be used to prevent drying of slants. Sterilize mineral oil in a hot air oven at 170°C for 1 hour. Add sufficient sterile mineral oil to cover the slants to 1 cm above the top of the agar. Subculture when needed by scraping growth from the slant. Strains maintained in pure culture in this manner will usually survive for several years.

2. Long-term storage

Bacterial cultures may be stored frozen or lyophilized in a variety of suspending media formulated for that purpose. There are many formulations of suspending medium, but, in general, skim milk, serum-based media, or polyvinylpyrrolidone (PVP) medium is used for lyophilization, and skim milk, blood, or a rich buffered broth such as Trypticase soy broth with 15% to 20% reagent grade glycerol is used for freezing.

Frozen storage method (ultralow freezer, -70°C; or liquid nitrogen freezer, -196°C)

Vibrios may be stored indefinitely if they are maintained frozen at -70°C or below.

- 1) Inoculate a TSA or HIA slant (or other noninhibitory, salt-containing growth medium) and incubate overnight at 35° to 37°C.
- 2) Harvest cells from the slant and make a suspension in freezing medium.
- 3) Dispense suspension into cryovials (freezing vials specially designed for use at very low temperatures). Caution: Do not use glass ampoules for freezing in liquid nitrogen because they can explode upon removal from freezer.
- 4) Rapidly freeze the suspension by placing the sealed vials in an alcohol-dry ice (frozen CO₂) bath until frozen. Transfer the frozen vials to a freezer.

Recovery of cultures from frozen storage

- 1) Place frozen cultures from the freezer on dry ice or into an alcohol-dry ice bath and transfer to a laboratory safety cabinet or to a clean area if a cabinet is not available.
- 2) Using a sterile loop, scrape the topmost portion of the culture and transfer to growth medium, being careful not to contaminate the top or inside of the vial.
- 3) Reclose vial before the contents completely thaw, and return vial to the freezer. With careful technique, transfers can be successfully made from the same vial several times.

Lyophilization

Vibrios may be successfully stored after lyophilization (freeze-drying). Freeze-drying involves the removal of water from frozen bacterial suspensions by sublimation under reduced pressure. Freeze-dried cultures of vibrios are best maintained at 4°C.

B. Transport and Shipment of Cultures and Specimens

1. Regulating organizations

The United Nations Committee of Experts on the Transport of Dangerous Goods develops recommended procedures for the safe transport of dangerous goods. The International Civil Aviation Organization (ICAO) has used these recommendations as the basis for developing regulations for the safe transportation of dangerous goods by air. The regulations of the International Air Transport Association (IATA) contain all the requirements of the ICAO Technical Instructions for the Safe Transport of Dangerous Goods. However, IATA has included additional requirements that are more restrictive than those of ICAO. Member airlines of the IATA have adopted the use of the IATA regulations governing dangerous goods, and shippers must comply with these regulations in addition to any applicable regulations of the state of origin, transit, or destination.

The shipment of infectious agents or diagnostic specimens by air must comply with local, national, and international regulations. International air transport regulations may be found in the ICAO publication entitled, "Dangerous Goods Regulations." This reference is published annually in January. The regulations may change from year to year. Obtain a copy of the IATA regulations in English, Spanish, French, or German from one of the regional offices listed below.

Orders from North, Central and South America, Asia, Australia and the Pacific:

Publications Assistant	Telephone: (514) 844-6311
Intl. Air Transport Assn.	FAX: (514) 844-5286
2000 Peel Street	Telex: 05-267627
Montreal, Quebec	Cable: IATA MONTREAL
Canada H3A 2R4	Teletype: YULTPXB

Orders from Europe, Africa, and the Middle East:

Publications Assistant	Telephone: (22) 799.25.25
Intl. Air Transport Assn.	FAX: (22) 798.35.53
IATA Centre	Telex: 415586
Route de l'Aéroport 33	Cable: IATA GENEVA
P.O. Box 672	Teletype: GVATPXB
CH-1215 Geneva 15 Airport	
Switzerland	

2. Guidelines for packaging and labeling infectious substances

The following guidelines for packaging infectious substances are taken from the 1992 IATA publication "Dangerous Goods Regulations." They are presented as an example of acceptable packaging procedures for infectious materials. However, they may not reflect current national, state, or IATA requirements for packaging and labeling for infectious substances. For current information on packaging and labeling requirements, consult the appropriate national and state regulations and the current-year IATA publication entitled "Dangerous Goods Regulations."

Persons who ship infectious agents or diagnostic specimens must comply with all local and international regulations pertaining to the packaging and handling of these items. They must ensure that specimens arrive at their destination in good condition and that they present no hazard to persons or animals during shipment.

Packaging guidelines for etiologic agents

The inner packaging must include

- An inner watertight primary container
- A watertight, impact-resistant secondary container

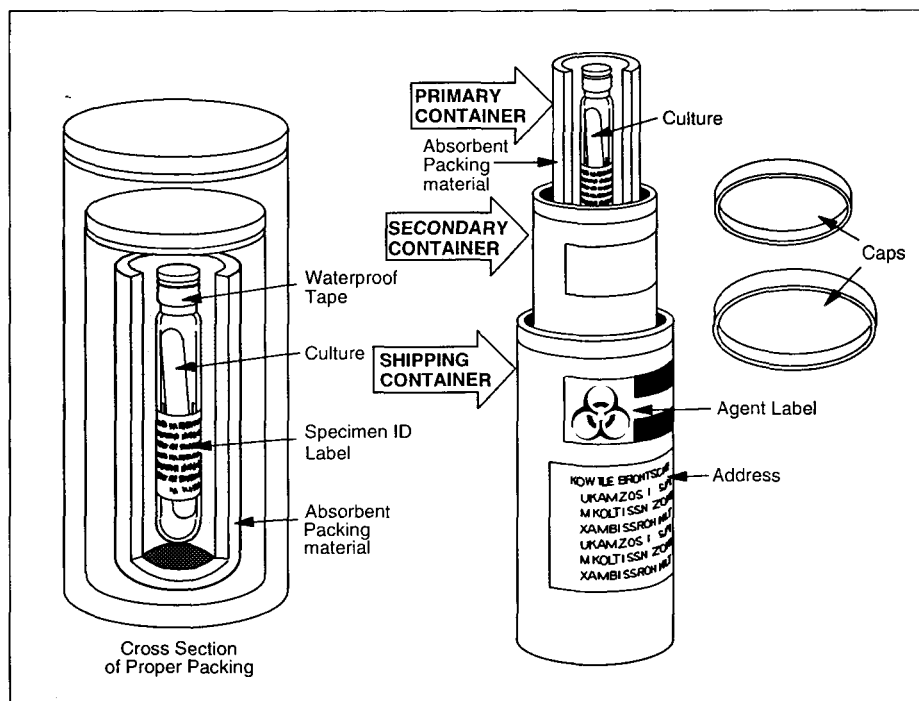


Figure XII-2. Packaging requirements for shipment of etiologic agents.

- Absorbent material between the primary container and the secondary container. If multiple primary containers are placed in a single secondary packaging, they must be wrapped individually to ensure that contact between them is prevented. The absorbing material, such as cotton wool, must be sufficient to absorb the entire contents of all primary containers.

The outer packaging must be of sufficient strength to adequately protect and contain the contents. The outer container must be at least 100 mm (4 inches) in its smallest overall external dimension. An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. Packages must be durably and legibly marked on the outside of the package with the address and telephone number of the consignee. A biohazard warning label must be affixed to the outside of the outer container, and must bear the inscription, "Infectious substance. In case of damage or leakage immediately notify public health authority." Figure XII-2 illustrates these packaging recommendations.

References

1. World Health Organization. *Manual for Laboratory Investigations of Acute Enteric Infections*. Geneva: World Health Organization, 1987; publication no. WHO/CDD/83.3 rev 1.
2. International Air Transport Association. Annual Publication. "Dangerous Goods Regulations." IATA Publications Office, Montreal, Quebec, Canada.