Chapter 10
Storage of Isolates

Shigella, *Vibrio cholerae*, or *Escherichia coli* serotype O157:H7 will usually remain viable for several days on solid medium held at ambient 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. Selection of a storage method depends on the length of time the organisms are to be held and the laboratory equipment and facilities available.

A. Short-term Storage

Blood agar base (BAB), tryptone soy agar (TSA), and heart infusion agar (HIA) are examples of good storage media for enteric organisms. Carbohydrate-containing media (e.g., Kligler iron agar or triple sugar iron agar) should not be used because acidic byproducts of metabolism quickly reduce viability. BAB, TSA, and HIA all contain salt, which enhances growth of *V. cholerae*. Nutrient agar should not be used for growth or storage of *V. cholerae* since it has no added salt.

When preparing storage medium, while the tubes are still hot after autoclaving, place them in a slanted position to provide a short slant and deep butt (2 to 3 cm). 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. 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 22° to 25°C in the dark.

Sterile mineral oil may also be used to prevent drying of slants. 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; there is no need to remove mineral oil to subculture. Strains maintained in pure culture in this manner will usually survive for several years.

B. 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 tryptone soy broth with 15% to 20% reagent grade glycerol is used for freezing.

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

Isolates may be stored indefinitely if they are maintained frozen at -70°C or below. Storage at -20°C is not recommended because some organisms will lose viability at this temperature.

- Inoculate a TSA or HIA slant (or other noninhibitory, salt-containing growth medium) and incubate overnight at 35° to 37°C.
- Harvest cells from the slant and make a suspension in freezing medium.
- 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 the freezer.
- Prepare an alcohol and dry ice bath by placing dry ice (frozen CO_2) in a leakproof metal container large enough to hold a metal culture rack, and add enough ethyl alcohol to submerge about half of the cryovial. Rapidly freeze the suspension by placing the sealed vials in the dry ice bath until frozen. Transfer the frozen vials to a freezer. If there is no dry ice available, a container of alcohol may be placed in the freezer overnight and then used to quick-freeze vials.

Recovery of cultures from frozen storage

- Place frozen cultures from the freezer on dry ice or into an alcohol and dry ice bath and transfer to a laboratory safety cabinet or to a clean area if a cabinet is not available.
- 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.
- 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

Most organisms 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 are best maintained at 4°C.