Vibrio cholerae O1/O139 Preservation Methods

GLOBAL TASK FORCE ON CHOLERA CONTROL

Pure bacterial cultures of *Vibrio cholerae* (VC) can be stored for variable periods of time, depending on the storage method, the laboratory equipment or facilities available, and the downstream applications. Isolates must be carefully prepared for storage:

1. Select a confirmed VC colony and inoculate onto fresh non-selective media*.
2. Incubate at 35 ± 2 °C for 18-24 hours.
3. Check culture purity by ensuring the presence of well-isolated individual colonies without contaminants. Observe the isolate's appearance, then perform an oxidase test and serology to confirm.
4. Regardless of the preservation method used work aseptically, all tubes must be carefully labelled with permanent marker or barcodes, and recorded in appropriate registers.

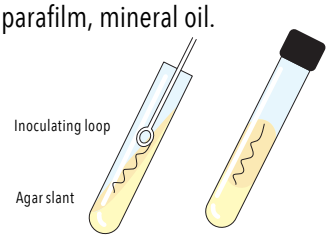
NON-SELECTIVE SLANTS

Up to 3 months ‡

Purpose: Laboratory stock strains.

Storage conditions: 22-25 °C in the dark.

Materials: Non-selective media slant*, loop, caps, sealing tape or parafilm, mineral oil.



SOP:

1. Inoculate agar surface using a loop from a fresh plate of pure culture, avoid going to the bottom of the slant where liquid can collect.
2. Replace lid loosely.
3. Incubate at 35 ±2 °C for 18-24 hours.
4. Check growth.
5. A layer of mineral oil can be added, before tightly closing the lid, to create an airtight seal to avoid drying out.

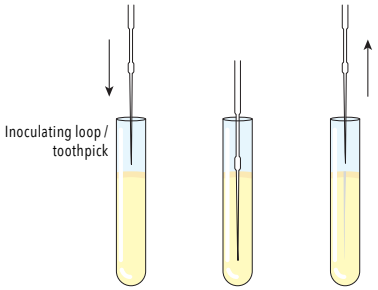
NON-SELECTIVE STABS

Up to 1 year ‡

Purpose: Sample shipping and laboratory stock strains.

Storage conditions: 22-25 °C in the dark.

Materials: Semi-solid non-selective stab*, sterile loop or toothpick, caps and sealing tape or parafilm, mineral oil.



SOP:

1. Inoculate stab from a fresh plate of pure culture, insert a heavily loaded loop or toothpick into the tube and push through the agar until it is approximately 1 cm from the bottom.
2. Remove the inoculation tool.
3. Replace lid loosely.
4. Incubate at 35 ±2 °C for 18-24 hours.
5. Check growth.
6. A layer of mineral oil can be added, before tightly closing the lid, to create an airtight seal to avoid drying out.

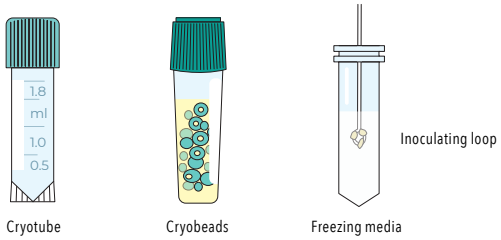
ULTRA LOW TEMPERATURE FREEZING

Indefinitely ‡

Purpose: Long-term storage of strains, research or stock maintenance.

Storage conditions: ≤-70 °C.

Materials: Liquid media**, sterile glycerol stock solution, cryotube, freezer box, ≤-70 °C freezer, optional – cryobead vial, vortex.



SOP:

1. To prepare freezing media, add stock glycerol to liquid media to a final concentration of 10% glycerol† in a cryotube.
2. Inoculate a heavy load from a fresh plate of pure culture into prepared tube.
   Or if using cryobeads: follow the manufacturer's instructions. Add the bacterial culture to the cryobead vial.
3. Mix or vortex well.
4. Record number of freezer box and position of tube in the box.
5. Immediately place into ≤-70 °C freezer.

* Non-selective agar, such as Mueller Hinton (recommended), Brain Heart Infusion, or Trypticase soy may be used.

** Appropriate liquid culture medium such as Trypticase soy broth, Brucella broth or Luria Bertani broth may be used.

† Use of up to 30% glycerol has been reported however ATCC recommends a final concentration of 10% https://www.atcc.org/resources/culture-guides/bacteriology-culture-guide

‡ Storage time may depend on number of times samples are opened or number of freeze thaw cycles.

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