



Gelatin Phosphate Salt Agar (GPS Agar)

M921

Gelatin Phosphate Salt Agar is used for characterization of *Vibrio cholerae* from food.

Composition**

Ingredients	Gms / Litre
Gelatin	10.000
Sodium chloride	10.000
Dipotassium phosphate	5.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40 grams in 1000 ml warm distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Vibrio cholerae is a non-halophilic *Vibrio*, which cannot grow in media with a concentration of sodium chloride greater than 5- 6% and is able to grow in media lacking NaCl (1). Human disease is associated with ingestion of contaminated water or food.

V.cholerae is the etiological agent of a secretory diarrhea spread by the faecal-oral route. The most critical virulence factor of *V.cholerae* is CT, which is responsible for the main symptom of the cholera disease (2). Gelatin Phosphate Salt Agar is a non-selective medium formulated as per APHA (3) and used for plating enrichment cultures of *V.cholerae* obtained from seafoods or vegetables.

Gelatinase enzyme producing *Vibrio* 's degrade gelatin and form small colonies, which are transparent with a cloudy halo. Gelatinase negative organisms show a satellite growth and may surround the colonies of *V.cholerae* on this medium. Dipotassium phosphate buffers the medium while sodium chloride maintains osmotic balance.

For enrichment and plating weigh 25 grams of sample in two jars of 500 ml capacity. Blend the vegetables or seafood into small pieces. Add 225 ml of GPS Broth to one jar and the same quantity of Alkaline Peptone Water (M618) to another and mix both the samples. Incubate each broth at $35 \pm 2^\circ\text{C}$ for up to 8 hours. If desired, then enumerate the bacterial count by MPN technique. Prepare the dried plates of media like TCBS Agar (M189) and another like GPS Agar (M921), nonselective media like (Cellobiose Polymyxin Colistin (CPC) Agar (M1241) and Sodium Dodecyl Sulphate Polymyxin Sucrose (SDS) Agar (M1155) may be also included. From the surface growth of each broth culture, inoculate two plating media by streaking. Incubate overnight at 35°C for 18 to 24 hours. From each plated medium, subculture to TN Agar (M950) slants or Motility Test Medium (M260) stabs and incubate overnight at $35^\circ \pm 2^\circ\text{C}$.

Quality Control

Appearance

Off white to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured, clear to slightly opalescent gel

Reaction

Reaction of 4% w/v aqueous solution at 25°C . pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

M921: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Colony characteristics
<i>Vibrio cholerae</i> ATCC 15748	50-100	good-luxuriant	transparent colonies with a cloudy halo

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label

Reference

- 1.Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
- 2.Bruno Gomez-Gil and Ana Roque, Isolation, Enumeration and Preservation of the Vibrionaceae, Thompson F. L., Austin B. and Swings J., The Biology of Vibrios, ASM press.
- 3.Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

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