Gelatin Phosphate Salt Agar (GPS Agar) M921

Intended Use:

Recommended for cultivation and characterization of *Vibrio cholerae* from food.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 grams in 1000 ml warm purified/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 (2). 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 (1). Gelatin Phosphate Salt Agar is a non-selective medium formulated as per APHA (5) 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.

Type of specimen

Food samples

Specimen Collection and Handling

For enrichment and plating weigh 25.0 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 ± 2°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), selective 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° ± 2°C.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. It require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain the specified pH.
2. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within expiry period when stored at the recommended temperature.

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

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. 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.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology,1996, 14th Edition, Churchill Livingstone
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

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