



Gelatin Salt Agar

M1148

Intended Use:

Recommended for cultivation and differentiation of *Vibrio* species from food.

Composition**

Ingredients	Gms / Litre
Gelatin	15.000
Peptone	4.000
Yeast extract	1.000
Sodium chloride	30.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Vibrio's are fairly easy to isolate from both clinical and environmental material, though some species may require growth factors and/or vitamins. Media can be made selective for *Vibrio*'s by adding appropriate selective agents (1). High concentrations of NaCl and alkaline pH have also been used to select certain *Vibrio* species, based on the ability of most *Vibrios* to grow at pH values above 8.0 and at 3% or higher concentrations of NaCl. *Vibrio* species which require high sodium chloride concentration for growth are called Halophilic or Halophiles. Gelatin Salt Agar is used to screen isolates for salt tolerance (6). As only halophilic *Vibrio* species grow on Gelatin Salt Agar, it is recommended to inoculate Gelatin Agar (M920) in addition (2). An opaque halo is observed around growth of gelatinase positive organisms.

Peptone and yeast extract provides essential nutrients required for growth of *Vibrio* species. Gelatin serves as a substrate for gelatinase reaction. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

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 requires 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 the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel and 1.5% Gelatin gel

Colour and Clarity of prepared medium

Light yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.5% 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	Growth	Gelatinase reaction
<i>Vibrio cholerae</i> ATCC 15748	luxuriant	positive reaction, opaque halo around the colony
<i>Vibrio parahaemolyticus</i> ATCC 17802 (00037*)	luxuriant	positive reaction, opaque halo around the colony
<i>Vibrio vulnificus</i> ATCC 29306	luxuriant	positive reaction, opaque halo around the colony
<i>Vibrio mimicus</i> ATCC 33653	luxuriant	positive reaction, opaque halo around the colony

Key : *Corresponding WDCM numbers.

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*, F.L. Thompson, B. Austin and J. Swings, The Biology of *Vibrios*, ASM press.
2. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.
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.
6. Smith Jr. H. L. and Goodner K., 1958, J. Bacteriol., 76:66

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