Gelatin Agar

M920

Intended Use:

Recommended for cultivation and identification of *Vibrio* species.

Composition**

Ingredients	Gms / Litre
Gelatin	30.000
Tryptone	10.000
Sodium chloride	10.000
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 warn preheated 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Members of the genus *Vibrio* are facultative anaerobes capable of both respiratory and fermentative metabolism. The natural habitat for *Vibrio* species is aquatic, in both fresh water and salt water. The growth and biochemical reactivity of most species are enhanced in different test media supplemented with 1- 2 % sodium chloride. *Vibrios* 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 (2). High concentrations of NaCl and alkaline pH have also been used to select certain *Vibrio* species, based on the ability of most *Vibrio's* to grow at pH values above 8.0 and at 3% or higher concentrations of NaCl. Gelatin Agar is formulated in accordance with APHA (5) for the cultivation and characterization of *Vibrio* species from foods and faeces. Clinical specimens must be obtained early in the disease as possible because the duration of excretion of the pathogen is short.

Weigh 25 grams of sample such as seafood or vegetables either blended or cut into small pieces and add into 2 flasks. Add 225 ml Alkaline Peptone Water (M618) to one flask and 225 ml of Glucose Phosphate Broth (M070) in another flask. Mix well. Incubate at 35° ± 2°C for 6 to 8 hours. Inoculate one loopful from each flask on the non-selective Gelatin Agar.

V.cholerae appear transparent and usually have a characteristic cloudy zone around colony, which becomes more definite after few minutes of refrigeration. When these colonies are viewed in oblique light they appear iridescent green to bronze coloured and finely granular.

Type of specimen

Food samples, Water samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

For water samples, follow appropriate techniques for sample collection and processing as per guidelines (1).

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 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 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 3.0% Gelatin gel

Colour and Clarity of prepared medium

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 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Gelatin liquefaction
Vibrio cholerae ATCC 15748	50-100	luxuriant	>=50%	positive reaction, clear zone around the colony within 24-48 hours
Vibrio parahaemolyticus ATCC 17802 (00037*)	50-100	luxuriant	>=50%	positive reaction, clear zone around the colony within 24-48 hours

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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. 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.
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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