Vibrio Vulnificus Agar (VVA)

M1878

Intended Use:

Recommended for identification of *Vibrio* in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Peptone	20.000
Sodium Chloride	30.000
Cellobiose	10.000
Bromothymol blue	0.060
Agar	25.000
Final pH (at 25°C)	8.20±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 85.06 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

V. vulnificus has been reported to be an important cause of death due to seafood consumption or after wound infections originating from marine environment. *Vibrio* species in general are alkalophilic and grow well in the presence of relatively high levels of bile salts. This necessitates the used of formulations with alkaline pH for their isolation and identification. Different methods used for the confirmation of *Vibrio* species include physical, biochemical and serological assays(6). *V.vulnificus* resembles *V. parahaemolyticus* on TCBS agar, but can be differentiated by several biochemical reactions, including beta-galactosidase activity. Identification using oligo nucleotides have also been recommented for the specific identification of the species (3). The oligonucleotide scheme includes both MPN and direct plating methods followed by hybridization with DNA probes for colony identification. CPC Agar Base (M1241F), Alkaline peptone water (M618) and TCBS Agar (M870S) are the most used formulations for the isolation of *Vibrios.*

Vibrio vulnificus Agar is used for the identification of *Vibrio vulnificus* from food samples through oligonucleotide analysis in accordance with FDA BAM, 1998 (2). Peptone provides necessary nitrogenous compounds to the medium. Cellobiose acts as the fermentable carbon source. Sodium chloride maintains the osmotic equilibrium of the medium. Bromothymol blue acts as the indicator dye and agar as the solidifying agent.

Prepare a 1:10 dilution of the sample in phosphate buffered saline (PBS) dilution water. Blend it for 60 sec. Prepare appropriate dilutions of the sample (if required) and pipet 0.1ml of the respective dilutions onto labeled VVA plates. Incubate the plates for 18-24 h at 35 ±2°C. Relatively large (1-2 mm) yellow opaque colonies (fried egg appearance) are typical of *V. vulnificus* on VVA. These colonies can be proceeded for Enumeration of *V. vulnificus* by DNA gene probe(2).

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,8). 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. Some isolates may show less growth due to nutritional variations.

2. Further biochemical and serological tests must be carried out for complete 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 light green homogeneous free flowing powder

Gelling
Firm, comparable with 2.5% Agar gel

Colour and Clarity of Prepared Medium
Green coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 8.51% w/v aqueous solution at 25°C. pH : 8.20±0.2

pH
8.00-8.40

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Vibrio vulnificus ATCC 29306	50-100	luxuriant	>=50%	Yellow opaque colonies (fried egg appearance)

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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3. Hill, W. E., Keasler, S.P., Trucksess, M.W., Feng, P ., Kaysner, C.A. and Lampel, K.A. 1991. Appl. Environ. Microbiol, 57: 707-711.
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5. 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.
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7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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