Vibrio Vulnificus Agar (VVA)

M1878

Vibrio Vulnificus Agar (VVA) is used for identification of *Vibrio* species 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 distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. 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(1). *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(2). 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 (3). 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(3).

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 Response
M1878: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

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

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

Reference
1.McPherson, V. L., Watts, J. A., Simpson, L. M . and Oliver, J. D. 1991. Microbios, 67: 141-149.
2.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.
3.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.

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