Saline Nutrient Agar for Vibrio

M2086I

Intended use

Recommended for isolation of *Vibrio* species from food, animal feeding stuff and environmental samples in the area of food production and food handling. The composition and performance criteria of this medium are as per the specifications laid down in ISO 21872-1: 2017 (E).

Composition**

ISO 21872-1:2017(E)-Saline Nutrient Agar

Ingredients	g / L
Meat extract	5.000
Peptone	3.000
Sodium chloride	10.000
Agar	8-18
Final pH after sterilization (at 25°C)	7.2±0.2

Saline Nutrient Agar for Vibrio **M2086I**

Ingredients	g / L
HM extract #	5.000
Peptone	3.000
Sodium chloride	10.000
Agar	15.000
Final pH after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Meat extract

Directions

Suspend 33.0 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Alternatively the medium can be dispensed into tubes to prepare slants.

Principle And Interpretation

Vibrio's are fairly easy to isolate from both clinical and environmental materials, though some species may require growth factors and /or vitamins. *Vibrio parahaemolyticus* is the leading cause of bacterial diarrhea associated with the consumption of contaminated food products. Media can be made selective for *Vibrio*'s by adding appropriate selective agents (1). This medium is recommended by ISO to isolate *Vibrio* species from food, animal feeding stuff and environmental samples from areas in food production and food handling (1).*Vibrio furnissii* 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 (1).

Peptone and HM extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Food and animal feeding stuffs, environmental samples from areas in food production and food handling.

Specimen Collection and Handling:

For food and animal feeds, environmental samples in area of food production and food handling, follow appropriate techniques for sample collection and processing as per guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Processesing : ISO 21872-1:2017 (E) (1)

Primary Selective enrichment : 25gm or 25ml of test portion in 225ml ASPW, temperature depends upon the target *Vibrio* species and state of product like deep frozen or fresh for 6 h ± 1 hour (For *V. parahaemolyticus* & *V. furnissii* at 41.5± 1°C in fresh foods and 37± 1°C for deep frozen ,dried or salted products, For *V.vulnificus* at 37± 1°C for all product states).

Secondary Selective enrichment : Transfer 1 ml of culture from primary enrichment broth to 10ml of ASPW (sample is not agitated before taking the aliquot). Incubate the ASPW at 41.5 °C ± 1 °C and/or 37 °C ± 1 °C for 18 h ± 1 hour.

Isolation and identification : The cultures obtained in the ASPW are transfered on TCBS Agar (M189), incubate at 37 °C ± 1 °C for 24 h ± 3 hour, for development of well-isolated colonies. For the second selective medium, examine for the presence of colonies, which, according to their characteristics, may be considered as possible isolates of *V. parahaemolyticus, V. vulnificus,* and/or *V. furnissii.*

Confirmation : By molecular PCR and/or biochemical approaches. For biochemical testing, inoculate the colonies selected onto the surface of plates of Saline Nutrient Agar (M2086I). Incubate at 37 °C ± 1 °C for 24 h ± 3 hour. From these isolated colonies are inoculated in Arginine Dihydrolase Saline Broth (M1644I) and/or L- Lysine Decarboxylase Saline Broth (LDC) (M1778I), incubate at 37 °C ± 1 °C for 24 h ± 3 hour.

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. Further biochemical test to be required to fully distinguish these species from each other and from non-pathogenic *Vibrio* spp.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.3 % 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 37 ± 1°C for 24 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery Productivity
Vibrio parahaemolyticus NCTC 10885 (00185*)	50-100	good-luxuriant	>=70%
Vibrio furnissii NCTC 11218 (00186*)	50-100	good-luxuriant	>=70%
Vibrio vulnificus ATCC 27562 (00139*)	50-100	good-luxuriant	>=70%
Vibrio mimicus ATCC 33653	50-100	good-luxuriant	>=70%
Vibrio alginolyticus ATCC 17749	50-100	good-luxuriant	>=70%

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 (2,3).

Reference

1. Microbiology of the food chain- Horizontal method for the determination of *Vibrio* spp.-Part 1:Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*, ISO 21872-1:2017 (E).
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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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