High Salt Nutrient Agar

M1218

Intended Use:

Recommended for isolation and cultivation of salt tolerant *Vibrio* species. The composition and performance criteria of this media is as per the specifications laid down in ISO/DIS 8914:1990

Composition**

Ingredients	g / L
Peptone	5.000
HM extract #	5.000
Sodium chloride	30.000
Agar	15.000
Final pH (at 25°C)	8.5±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Meat extract

Directions

Suspend 55 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 dispense as desired.

Principle And Interpretation

Vibrios 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 *Vibrios* by adding appropriate selective agents (1). High concentrations of NaCl and alkaline pH have also been used to select certain *Vibrio* species, based on their ability to grow at pH values above 8.0 and at 3% or higher concentrations of NaCl.

Vibrio cholerae 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 (2). High Salt Nutrient Agar is recommended for the isolation, cultivation and confirmation of salt-tolerant *Vibrio* species in products intended for human consumption or animal feeding stuffs in accordance with ISO Committee under specification ISO/DIS 8914:1990 (3).

HM extract and peptone are sources of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium and provides the essential ions.

Type of specimen

Clinical samples: faeces; Food samples

Specimen Collection and Handling

Inoculate 25 grams of test portion to 225 ml Salt Polymyxin Broth Base (M821I) and 225 ml Alkaline Peptone Water (M618I). Incubate the two broths at 35-37°C for 7-8 hours. After incubation, inoculate a loopful from M821I onto TCBS Agar (M189), Tryptone Sucrose Tetrazolium Agar Base (M1217) and High Salt Nutrient Agar (M1218). Repeat the plating procedure for M618I. Incubate the plates at 35-37°C for 20-24 hours. Confirm presumptive *Vibrio* colonies by performing the biochemical tests. This can be performed by inoculation into High Salt Peptone Yeast Extract Agar (M1219). This medium can be used to differentiate between aerobic and anaerobic growth.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Further recovery from this enriched broth onto selective media is required.
2. Biochemical characterization is carried out from pure isolates 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 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 5.5% w/v aqueous solution at 25°C. pH : 8.5±0.2

pH
8.30-8.70

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

Organism	Inoculum (CFU)	Growth	Recovery
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%
Vibrio parahaemolyticus ATCC 17802 (00037*)	50-100	good-luxuriant	>=50%

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 inorder 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. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
2. Bruno Gomez-Gil and Roque A., Isolation, Enumeration and Preservation of the *Vibrionaceae,* Thompson F. L., Austin B. and Swings J., The Biology of *Vibrios*, ASM press.
3. International Organization for Standardization (ISO), 1990, Draft ISO/DIS 8914:1990
4. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, American Society for Microbiology, Washington, D.C.
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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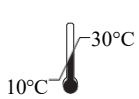
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

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