High Salt Nutrient Agar

M1218

High Salt Nutrient Agar is recommended for the isolation, cultivation and confirmation of salt tolerant *Vibrio* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Meat 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

Directions

Suspend 55 grams in 1000 ml 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).

Meat extract, L-cysteine hydrochloride and peptic digest of animal tissue are sources of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium and provides the essential ions.

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.

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

M1218: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

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

Storage and Shelf Life

Store below 30°C in tighty closed container and prepared medium at 2-8°C. Use before expiry period on the label.

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 8914:1990P

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