Technical Information

Dihydrolase Broth Base

Product Code: DM 1915

Application: - Dihydrolase Broth Base is used for studying dihydrolase reaction of *Vibrio parahaemolyticus* .

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Yeast extract	6.000
Dextrose	2.000
Sodium chloride	30.000
Bromo cresol purple	0.032
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Vibrios are easy to isolate from both clinical and environmental material, though some species may require growth factors and /or vitamins. *Vibrio parahaemolyticus* is the major cause of bacterial diarrhoea due to with the consumption of contaminated food products [1]. Dihydrolase Broth Base formulated as per APHA [2] is used for studying dihydrolase reaction of *V. parahaemolyticus* for differentiating from other *Vibrio species.* The medium is supplemented with L-Arginine as a substrate for dihydrolase reaction [3, 4]. L-Arginine is converted to putrescine by the dihydrolase enzyme; however putrescine is also formed from arginine by the decarboxylase system as well. In the decarboxylase system, L-Arginine undergoes decarboxylation to yield agmatine. Agmatine is then catabolized by the enzyme agmatine dihydrolase to putrescine, CO_2 and ammonia by way of an intermediate compound monocarbaminyl putrescine [5]. Thus, because of production of amine like putrescine in the medium the pH is increased [6] changing the colour of the indicator from yellow to purple. Bromocresol purple is the pH indicator in the medium, which turns purple from yellow at alkaline pH. For confirmation, it is suggested to inoculate a basal medium tube, which does not contain L-Arginine. Alkalinization of the surface of the medium may be caused by exposure to air, so a dihydrolase negative organism may be misidentified as positive. It is therefore recommended to protect the inoculated tubes from air with overlay of sterile mineral oil. Peptic digest of animal tissue and yeast extract provide nitrogenous nutrients to support bacterial growth. Dextrose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium.

Methodology

Suspend 43.03 grams of powder media in 1000 ml distilled water. Shake well & heat, if necessary to dissolve the medium completely. Divide in 2 parts. Add 0.5% L-Arginine to first portion. Use second portion as control. Dissolve completely and dispense 3.0 ml into 13 mm x 100 mm screw cap tube. Sterilize by autoclaving at 115°C for 15 minutes.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured, clear solution without any precipitate

Reaction
Reaction of 4.3% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH range
6.60-7.00

Cultural Response/Characteristics
DM1915: Cultural characteristics observed with added 0.5% L-Arginine after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Arginine dihydrolase
Enterobacter aerogenes ATCC 13048	50-100	Good-Luxuriant	negative reaction,yellow colour
Vibrio cholerae ATCC15748	50-100	Good-Luxuriant	negative reaction,yellow colour
Vibrio parahaemolyticus ATCC 17802	50-100	Good-Luxuriant	positive reaction, purple colour

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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. Speck M. L., (Eds.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd Ed., APHA, Washington, D.C.
3. Moeller V., 1955, Acta Pathol. Microbiol. Scand., 36:158.
4. Slade H. D. and Slamp W. C., 1952, J. Bacteriol., 64:455.
5. Oginsky E. L. and Gehrig R. F., 1953, J. Biol. Chem., 204:721.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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