Dihydrolase Broth Base

Intended Use

Recommended for studying dihydrolase reaction of *Vibrio parahaemolyticus*

Principles of the Procedure

Vibrios are fairly easy to isolate from both clinical and environmental material, though some species may require growth factors and /or vitamins. *Vibrio parahaemolyticus* is the leading cause of bacterial diarrhoea associated with the consumption of contaminated food products (1). Dihydrolase Broth Base is formulated as per APHA (9) and is used for studying dihydrolase reaction of *V. parahaemolyticus*.
Dextrose is utilized by *Vibrio* species where there is drop in pH indicated by Bromocresol purple resulting in yellow colour. The medium is supplemented with L-Arginine as a substrate for dihydrolase reaction (5,8). L-Arginine is converted t 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, CO2 and ammonia by way of an intermediate compound monocarbaminyl putrescine (6). Thus, because of production of amine like putrescine in the medium the pH is elevated (4) 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. Peptone and yeast extract provide nitrogenous nutrients to support bacterial growth. Dextrose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium.

Formula / Liter

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	6.000
Dextrose (Glucose)	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 p s

Directions

Suspend 43.03 grams in 1000 ml purified / distilled water. 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 10 lbs pressure (115°C) for 15 minutes.

Quality Control Specifications

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	6.60-7.00
Cultural Response	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
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	negative reaction,yellow colour
Vibrio parahaemolyticus ATCC 17802 (00037*)	50-100	good-luxuriant	negative reaction, yellow colour

Key : (*) Corresponding WDCM numbers

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