Technical Information

Glucose Salt Teepol Broth (Twin Pack)

Product Code: DM 1621

Application: - Glucose Salt Teepol Broth is used for enrichment of *Vibrio parahaemolyticus* and marine isolates.

Composition**

Ingredients	Gms / Litre
Part A	-
Peptic digest of animal tissue	10.000
Beef extract	3.000
Sodium chloride	30.000
Glucose	5.000
Methyl violet	0.002
Part B	-
Teepol	4.000
Final pH (at 25°C)	8.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Glucose Salt Teepol Broth is a special media used to enrich Vibrio parahaemolyticus from sea foods [4] and also to enumerate the bacteria by MPN technique [1].

V.parahaemolyticus is a gram-negative marine bacterium, which causes seafood-borne gastroenteritis in humans [2]. Fujino and co-workers were the first to isolate Vibrio parahaemolyticus as a causative agent of food-borne gastroenteritis, outbreak in Japan [3].

Peptic digest of animal tissue and beef extract provide essential nitrogenous nutrients and the high percentage of sodium chloride (3%) helps for the better enrichment of halophilic V.parahaemolyticus. Glucose is utilized while teepol inhibits the growth of gram-positive organisms. The test sample should be held under moderate refrigeration (about 7 to 10°C) and should be analyzed as soon as possible, after collection as possible. This maximizes the survival and recovery of Vibrio's and reduces the tendency of overgrowth by indigenous marine microflora.

Weigh 50 gram of seafood sample into a blender. Add 450 ml of PBS (Phosphate Buffer Saline) dilution water and blend for 1 min at 8000 rpm. This constitutes the 1:10 dilution. Prepare 1:100, 1:1000, 1:10000 dilutions or higher if necessary in PBS. Inoculate 3 x 10 ml portion of the 1:10 dilution into 3 tubes containing 10 ml of enrichment broth i.e. Glucose Salt Teepol Broth in 2x concentration. This represents the 1-gram portion. Similarly inoculate 10 ml of single strength enrichment broth as above. If high numbers of V.parahaemolyticus are expected, the examination may start at the 1:10 dilution of the product [1]. After overnight incubation of Glucose Salt Teepol Broth at 35 ± 2°C, a loopful of culture from top 1 cm of the broth showing growth is streaked onto TCBS Agar (DM1189). After overnight incubation at 35 ± 2°C, V.parahaemolyticus colonies on TCBS Agar appear as round, green or bluish measuring 2.3 mm in diameter, while V.alginolyticus colonies are larger and yellow coloured. These colonies are further identified by biochemical characterization. For biochemical tests in identification of V.parahaemolyticus, V.cholera , and V.vulnificus , appropriate positive control organisms have to be inoculated.

When the blue green colonies are finally identified as V.parahaemolyticus, refer to the original positive dilution in the enrichment broth and apply the 3 tube MPN tables for final enumeration of the organism.

Methodology

Suspend 48 grams of Part A media in 1000 ml distilled water containing 4.0 ml of Part B medium. Shake well & heat gently to dissolve the medium completely. Dispense in tubes as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance
Part A : Cream to yellow homogeneous free flowing powder Part B : Colourless viscous liquid

Colour and Clarity of prepared medium
Yellow coloured, clear solution with a very sligh precipitat

Reaction
Reaction of 4.8% w/v aqueous solution with 0.4% Teepol at 25°C. pH : 8.8±0.2

pH range 8.6-9.0

Cultural Response/ characteristices
DM 1621: Cultural characteristices observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
Vibrio alginolyticus ATCC 17749	50-100	good
Vibrio parahaemolyticus ATCC 17802	50-100	good

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° in sealable plastic bags for 2-5 days.

Further Reading

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
2. Thompson F. L., T. Iida and Swings J., 2004, Biodiversity of Vibrios, Microbiol. Mol. Biol. Rev., 68: 403-43 1. 3.Fujino T., Okuno Y., Nakada D., Aoyama A., Fukai K., Mukai T. and Ueho T., 1953, Med. J. Osaka Univ., 4:299-304. 4.Akiyama S., Takizawa K., and Obara Y., 1964, Ann. Rep. Kanagawa Pref. Inst. Public Health, 13:7

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