Bromo Cresol Purple Azide Broth

M1212

Intended use

Bromo Cresol Purple Azide Broth is used for the confirmation of the presence of faecal Streptococci in water and wastewater.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	10.000
D-Glucose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.700
Potassium dihydrogen phosphate	2.700
Sodium azide	0.500
Bromocresol purple	0.032
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.93 grams in 1000 ml distilled water. Add 5 ml glycerol if desired. Heat, if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle And Interpretation

Enterococci are widely distributed and occur in different habitats. The Enterococci portion of the faecal *Streptococcus* group is a valuable bacterial indicator for determining the extent of faecal contamination of recreational surface waters. Studies indicate that swimming associated gastroenteritis is related directly to the quality of bathing water and that enterococci are the most efficient bacterial indicators of water quality (1, 2). Bromo Cresol Purple Azide Broth formulated as per Hajna & Perry (3) is used for the confirmation of the presence of faecal streptococci in water and wastewater. This medium is used for testing water samples, after preliminary testing of water samples in Azide Dextrose broth (M345). Bromo Cresol Purple Azide Broth is recommended by APHA for enumerating faecal streptococci by the MPN technique (4).

Bromo Cresol Purple Azide Broth has dextrose (D-glucose) as the fermentable carbon source and bromocresol purple as an indicator. The colour change of the medium from purple to yellow indicates fermentation of dextrose (D-glucose) and subsequent acid production. According to Hajna, enterococcal dextrose fermentation is improved by the addition of glycerol (3). Tryptone and yeast extract supply nitrogenous compounds, sulphur, amino acids and trace ingredients. Sodium chloride maintains osmotic balance of the medium. Sodium azide inhibits the entire bacterial flora including those species that may have grown in the preliminary test media. Colour change to yellow with turbidity indicates and confirms the growth of Enterococci.

Type of specimen

Clinical samples - Water and waste water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(5) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic 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 guidleines should be followed while handling clincal specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations

1. Further biochemical tests must be carried out for further confirmation.

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

Light yellow to beige homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured, clear solution without any precipitate

Reaction

Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M1212: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Acid
Cultural Response			
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	positive reaction, yellow colour
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^3	inhibited	
Streptococcus agalactiae ATCC 13813	50-100	none-poor	negative reaction, no colour change
Streptococcus pyogenes ATCC 19615	50-100	none-poor	negative reaction, no colour change

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. Store below 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (6,7).

Reference

1. Cabelli V. J., 1983, EPA-600/1-80-031, U. S. Environmental Protection Agency, Cincinnati, Ohio.
2. Dufour A. P., 1984, EPA-600/1-84-004, U. S. Environmental Protection Agency, Cincinnati, Ohio.

3. Hajna A.A. and Perry C.A., 1943, Am. J. Publ. Health, 33:550.
4. Hajna A.A., 1951, Public Health Lab., 9:80-81.
5. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
7. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.

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