Glucose Azide Broth

M982

Intended Use:

Recommended for the enumeration of faecal Streptococci by MPN technique from water and sewage.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Yeast extract	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	5.000
Potassium dihydrogen phosphate	2.000
Dextrose (Glucose)	5.000
Sodium azide	0.250
Bromo cresol purple	0.030
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.28 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense 5ml amounts in 16 x150 mm test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For large inocula of 5 ml or more quantities, prepare double strength medium.

Principle And Interpretation

Fecal *Streptococcus* is a group of bacteria normally present in large numbers in the intestinal tract of warm-blooded animals other than human. The fecal *Streptococcus* group consists of a number of species of the genus *Streptococcus* such as *Streptococcus faecalis, Streptococcus faecium, Streptococcus avium, Streptococcus bovis, Streptococcus equines* and others. They have been used with fecal coliforms to differentiate fecal contamination from humans and from that of other warm-blooded animals. They tend to persist longer in the environment than faecal coliforms. Glucose Azide Broth is recommended by Hannay and Norton (3) for enumeration of faecal streptococci by MPN technique from water, sewage, foods and other materials suspected to be contaminated with sewage. Glucose Azide Broth is a highly nutritious medium due to its content of peptone, yeast extract and dextrose, which provide nitrogenous compounds, carbon, sulphur, amino acids and trace ingredients. Sodium chloride maintains osmotic balance of the medium. Sodium azide suppresses the growth of gram-negative organisms and thereby allows the selective growth of faecal Streptococci.

Type of specimen

Water sample

Specimen Collection and Handling:

Prior to inoculation, warm the tubes of Glucose Azide Broth to 44-45°C by heating in a water bath. Inoculate the tubes containing Glucose Azide Broth with heavy inocula from all the positive presumptive test tubes. MacConkey Broth purple (M083) is generally used for the presumptive test. Incubate inoculated Glucose Azide Broth tubes at 44-45°C for 24-48 hours. Tubes showing yellow colour change, due to acid production are subcultured onto Bile Esculin Azide Agar (M493) for confirming the presence of faecal streptococci (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. The media should be handled by trained personnel only. Ear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical tests must be carried out for 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 temmperature.

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 3.03% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural response

Cultural characteristics observed after an incubation at 35-37ºC for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Colour change to yellow
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	positive
Enterococcus hirae ATCC 8043 (00089*)	50-100	good-luxuriant	positive
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	negative
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	negative

Key : *- Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. 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.
2. Collee J. G., Duguid J. P., Fraser A. G., Marmion B. P., (Eds) Mackie and McCartney, Practical Medical Microbiology,1989, 13th Edition, Churchill Livingstone
3. Hannay C. L., Norton I. L., 1947, Proc. Soc. Appl. Bacteriol.1: 59
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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