PA Broth

M1186

Intended Use:

Recommended for the detection of presence & absence of coliform bacteria in water from treatment plants or distribution systems.

Composition**

Ingredients	g / L
Peptone	5.000
Tryptose	9.830
HM peptone B #	3.000
Lactose	7.460
Sodium chloride	2.460
Dipotassium hydrogen phosphate	1.350
Potassium dihydrogen phosphate	1.350
Sodium lauryl sulphate (SLS)	0.050
Bromo cresol purple	0.0085
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters
#- Equivalent to Beef extract

Directions

Suspend 30.51 grams in 1000 ml purified/distilled water or if desired, suspend 91.53 grams in 1000 ml distilled water to prepare a triple strength medium. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amount into screw-capped tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 12 minutes.
Note:Prepare triple strength medium for 100 ml sample.

Principle And Interpretation

Availability of clean water for bathing, drinking and cooking is critical for modern civilization. Different pathogens can be transmitted through water contaminated by faeces and other sources leading to diseases such as diarrhea, typhoid, cholera etc. Different strategies have been developed for bacteriological examination of water. Weiss and Hunter proposed a simplified procedure for the bacteriological examination of treated water (1). Later on the PA (Presence Absence) test was developed as a simplified version of the test based on the principle that coliforms and other bacterial indicators of pollution should not be found in 100 ml samples of treated water (2). Other aspects of PA test were studied by Clark et al (3). PA Broth has been included as a tentative standard in the Standard Methods for the Examination of Water and Wastewater (4) justified on the theory that a 100 ml sample of drinking water should not contain any coliform. The Presence Absence (PA) test for the coliform group is a simple modification of the multiple-tube procedures and provides a qualitative estimate of coliforms. This test is intended for use on routine samples collected from distribution system or water treatment plants. When PA test is positive, coliform densities can be determined quantitatively in repeat samples to indicate the magnitude of the contamination. PA test maximizes coliform detection in samples containing many organisms that could overgrow coliforms and cause problems in detection (5). The medium contains peptone, tryptose, HM peptone B which supply nitrogenous and carbonaceous compounds, long chain amino acids, growth factors and trace ingredients to the coliforms. Lactose serves as the fermentable carbohydrate and energy source for bacterial metabolism. Phosphates provide buffering action while sodium lauryl sulphate inhibits many organisms other than coliforms. Bromocresol purple is the pH indicator which turns yellow at acidic pH. Majority of the lactose fermenting coliforms utilize the lactose to form acid. This acidity is detected by the pH indicator (Bromocresol purple) which change colour from purple to yellow at acidic pH. The medium is used a triple strength medium when examining 100 ml samples. PA test is only a presumptive test for the presence of coliforms. Confirmation of these results must be achieved by using a medium like Brilliant Green Bile Broth (M121).

Type of specimen

Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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 guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. PA test is only a presumptive test for the presence of coliforms. Confirmation of these results must be achieved by using a medium like Brilliant Green Bile Broth (M121).

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 greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured clear solution without any precipitate

Reaction

Reaction of 3.05% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Colour of medium
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	yellow
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good-luxuriant	light yellow
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	-
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	yellow
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	no change (purple)

Key : *Corresponding WDCM numbers. # Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (5,6).

Reference

1. Weiss J.E. and Hunter C.A., 1939, J. Am. Water Works Assoc., 31 : 707.
2. Clark J. A., 1969, Can. J. Microbiol., 5: 771.
3. Clark J. A., Burger C.A. and Sabatinos L. E., 1982, Can. J. Microbiol., 28 : 100
4. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
6.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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