Technical Information

M-Azide Broth Base

Product Code: DM 2119

Application: - M-Azide Broth Base is a selective medium used for cultivation and enumeration of Enterococci from water samples using membrane filter technique.

Composition**

Ingredients	Gms / Litre
Tryptose	40.000
Yeast extract	10.000
Dextrose	2.000
Saccharose	100.000
Dipotassium phosphate	4.000
Sodium azide	0.400
Final pH (at 25°C)	7.1±0.2

Principle & Interpretation

Enterococci may be considered an essential part of the microflora of humans and animals. Because of its wide distribution, Enterococci can also occur in different food commodities, especially those of animal origin [1, 2]. The most important feature of this genus is their high level of antibiotic resistance. In water, bodies the acceptable level of contamination due to enterococci is very low. In 2004, *Enterococcus* species took the place of fecal coliform as the new federal standard for water quality at public beaches. Compare to fecal coliforms enterococci provide a higher correlation with many of the human pathogens often found in sewage [3].

M-Azide Broth was formulated by Slanetz, Bent and Bartely [4] and is especially recommended for the enumeration of Enterococci from water samples and other specimens using membrane filter technique. In this technique, a measured volume of the water sample is filtered through a membrane with a pore size small enough to retain the indicator bacteria to be counted. The membrane is then aseptically placed and incubated on a selective indicator medium (or sterile absorbent cotton pads saturated with the selective medium), so that the indicator bacteria grow into colonies on its upper surface [5].

Tryptose, yeast extract provide essential growth nutrients. Dextrose and saccharose are the fermentable carbohydrates. Sodium azide is used as selective agent, which inhibits gram-negative bacteria. Mallmann et al [6] reported that sodium azide exerts bacteriostatic effect on gram-negative bacteria allowing unrestricted growth of gram-positive cocci, particularly Enterococci. TTC imparts pink to red colour to the colonies. For membrane filter technique [7], 2.2 ml medium is added per absorbent pad. Using this medium, Slanetz et al observed better recovery of pure cultures of *Enterococcus faecalis* by membrane filter technique than MPN technique.

Methodology

Suspend 15.64 grams of powder media in 100 ml distilled water. Shake well & heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 1 ml of 1% 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC, MS2057). Mix well before dispensing.

Caution: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate

Reaction
Reaction of 15.64% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH range
6.90-7.30

Cultural Response/Characteristics
DM 2119: Cultural characteristics observed after an incubation at 35-37°C for 48 hours with added 1% 2,3,5 Triphenyl Tetrazolium Chloride (TTC, MS2057).

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane filter)
Escherichia coli ATCC 25922	>=10^3	inhibited	-
Enterococcus faecalis ATCC 29212	50-100	Good-Luxuriant	Pink to red

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: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Belzer, R., Inaug. Dissert., Univ. München, 1983.
2. Burkwall, M. K., Hartman, P. A., Appl. Microbiol., 12; 18-23 (1964).
3. Jin G., Jeng H., Bradford H., Englande A., 2004, Water Environ Res 76 (3): 245-55.
4. Slanetz L. W., Bent D. F. and Bartley C. H., 1955, Pub. Hlth. Rep., 70:67.
5.Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds) Mackie and McCartney, Practical Medical Microbiology, 1996, 14th edition, Churchill Livingstone.
6. Mallmann W. L., Botwright W. E. and Churchill E. S., 1941, J. Inf. Dis., 69:215.
7. MacFaddin J. F., 1985, Media for Isolation-Identification-Cultivation-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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