Technical Information

Ethyl Violet Azide Broth (EVA Broth)

Product Code: DM 1426S

Application: - Ethyl Violet Azide Broth (EVA Broth) is recommended for selective, confirmatory detection of Enterococci as an indicator of faecal pollution in water and other specimens.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Dextrose	5.000
Dipotassium phosphate	2.700
Monopotassium phosphate	2.700
Sodium chloride	5.000
Sodium azide	0.400
Ethyl violet	0.00083
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

EVA broth is based on the formulation of Litsky et al (1) and present medium is a modification (2) with the reduced amount of dextrose and increased dye concentration which is highly specific for Enterococci. The presence of Enterococci acts as a valuable index of faecal or sewage pollution in water (3). It is recommended by BIS for detection of faecal Streptococci (4). BIS has recommended EVA Broth for enumeration of Enterococci using MPN technique. EVA Agar can be prepared by adding 1.5% agar to EVA Broth before autoclaving. EVA Agar plates are used for isolation of Enterococci.

EVA Broth is used in conjunction with Azide Dextrose Broth (DM 1345). Larkin et al (5) used Azide Dextrose Broth as a presumptive medium and EVA Broth for the confirmation of the presence of Streptococci in frozen foods. They found that generally faecal Streptococci were recovered more consistently and in greater number than the coliforms and could be used in preference to coliforms as an indicator bacteria in frozen foods.

Casein enzymic hydrolysate and dextrose supply the necessary nutrients for the growth of Enterococci. Sodium azide and ethyl violet inhibit gram-negative bacilli and gram-positive spore formers.

Methodology

Suspend 35.8 grams of dehydrated media powder in 1000 ml distilled water. Mix thoroughly & heat, if necessary to dissolve the medium completely. Dispense in tubes in 10 ml amounts. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Warning: 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

Appearance

Cream to yellow coloured homogeneous free flowing powder

Colour and Clarity

Light amber coloured clear solution without any precipitate.

Reaction
Reaction of 3.58% w/v aqueous solution at 25°C. pH : 7.0±0.2

Ph Range
6.80-7.20

Cultural Response
DM 1426S: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Cultural Response		
Enterococcus faecalis ATCC 29212	50-100	luxuriant with purple button at the bottom of tube
Escherichia coli ATCC 25922	>=10^3	inhibited
Streptococcus pyogenes ATCC 19615	>=10^3	inhibited

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2-8°C.Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Litsky W., Mallmann W.L. and Fifield C.W., 1953, Am. J. Publ. Health, 43:873.
2. Litsky W., Mallmann W.L. and Fifield C.W., 1955, Am. J. Publ. Health, 45:104.
3. Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1985, Standard Methods for the Examination of Water and Wastewater, 16th ed., APHA, Washington D.C.
4. Bureau of Indian Standards IS : 5887 (Part II) 1976, reaffirmed 1986 (Second reprint December 1994).
5. Larkin, Litsky and Fuller, 1955, Appl. Microbiol., 3:98, 102, 104, 107.

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