Technical Information

Ethyl Violet Azide Broth (E.V.A. Broth)

Product Code: DM 1426

Application: - Ethyl Violet Azide Broth (E.V.A. Broth) is used as a selective and confirmative medium for detection of Enterococci and as an indicator of faecal pollution in water and other specimens.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	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

The present ethyl violet Azide Broth medium is a modification of medium developed by Litsky et al [3] in which the former has reduced amount of dextrose and increased dye concentration, making the present medium highly specific for Enterococci. The presence of Enterococci acts as a valuable index of faecal or sewage pollution in water [1]. E.V.A. Broth is used in conjunction with Azide Dextrose Broth (DM1345). Larkin et al [2] used Azide Dextrose Broth as a presumptive medium and E.V.A. 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.

Litsky et al [4] studied a variety of dyes and selective agents for Streptococci and developed a confirmatory medium using ethyl violet and sodium azide as selective agents. Combination of 0.0083gm% of ethyl violet dye and 0.04gm% of azide provided the best selective action favouring growth of Streptococci [4].

EVA Broth contains casein enzymic hydrolysate as source of carbon, nitrogen, vitamins and minerals. Dextrose is the fermentable carbohydrate. Sodium azide and ethyl violet inhibit gram-positive bacilli and gram-positive cocci other than Enterococci. Monopotassium and dipotassium phosphates buffer the medium. Sodium chloride provides osmotic balance.

Methodology

Suspend 35.8 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense in tubes in 10 ml amounts and 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 of the disposables.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured, clear solution in tubes

Reaction
Reaction of 3.5 8% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH range 6.80-7.20

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

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

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^{o} 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.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington D.C.
4. Larkin, Litsky and Fuller, 1955, Appl. Microbiol., 3:98, 102, 104, 107.

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