Ethyl Violet Azide Broth (E.V.A. Broth) M426

Intended Use:

Recommended for selective, confirmatory detection of *Enterococci* as an indicator of faecal pollution in water and other specimens .

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Dextrose (Glucose)	5.000
Dipotassium hydrogen phosphate	2.700
Potassium dihydrogen 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

Directions

Suspend 35.8 grams in 1000 ml purified/distilled water. Heat, if necessary 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.

Principle And Interpretation

Ethyl Violet Azide Broth is based on the formulation of Litsky et al (1) and the present medium is a modification of medium developed by Litsky et al (2) with reduced amount of dextrose and increased dye concentration, making the medium highly specific for *Enterococci.* The presence of *Enterococci* acts as a valuable index of faecal or sewage pollution in water (2). E.V.A. Broth is used in conjunction with Azide Dextrose Broth (M345). Larkin et al (3) 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 (2) 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* (3).

EVA Broth contains tryptone 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.

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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.
2. Use a heavy inoculum, EVA broth is not designed to support growth from dilute inocula (5)

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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured, clear solution in tubes

Reaction

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

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant with purple button at the bottom of tube
Streptococcus pyogenes ATCC 19615	>=10^4	inhibited

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1.Litsky W., Mallmann W.L. and Fifield C.W., 1955, Am. J. Publ. Health, 45:104.

2.Litsky W., Mallmann W.L. and Fifield C.W., 1953, Am. J. Publ. Health, 43:873.

3.Larkin, Litsky and Fuller, 1955, Appl. Microbiol., 3:98, 102, 104, 107.

4.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.

5.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.

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

7.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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