Ethyl Violet Azide Broth

Intended Use
Ethyl Violet Azide Broth (E.V.A. Broth) is used for selective, confirmatory detection of Enterococci as an indicator of faecal pollution in water and other specimens.

Summary
Ethyl Violet Azide Broth is based on the formulation of Litsky *et al.,* and the present medium is a modification of medium developed by Litsky *et al.,* 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. E.V.A. Broth is used in conjunction with Azide Dextrose Broth. Larkin *et al.,* 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.

Principle
Litsky *et al.,* 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. 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.

Formula*

Ingredients	**g/L**
Casein enzymic hydrolysate	20.0
Dextrose	5.0
Dipotassium phosphate	2.7
Monopotassium phosphate	2.7
Sodium chloride	5.0
Sodium azide	0.4
Ethyl violet	0.00083
Final pH (at 25°C)	7.0 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Water samples.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 35.80 g of the powder in 1000 mL purified / distilled water.
2. Heat if necessary,to dissolve the medium completely.
3. Dispense in tubes in 10 mL amounts and sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

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

Dehydrated Appearance: Cream to yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Light amber coloured, clear solution with bluish tinge in tubes.
Cultural Response: Cultural characteristics observed after an incubation at 35°C-37°C for 24-48 hours.

Organism (ATCC)	Growth
Escherichia coli (25922)	Inhibited
Enterococcus faecalis (29212)	Good (Purple button at the bottom of the tube)
Streptococcus pyogenes Strain Bruno (19615)	Inhibited

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. 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.
2. Litsky W., Mallmann W. L. and Fifield C. W., 1955, Am. J. Public Health, 45:104.
3. Litsky W., Mallmann W. L. and Fifield C. W., 1953, Am. J. Public Health, 43:873.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201050130500	Dehydrated Culture Media	500 g