



Ethyl Violet Azide Dextrose Agar

M1397

Ethyl Violet Azide Dextrose Agar is used for detecting and confirming Streptococci and as confirmative medium for faecal pollution indication 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
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

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 .

Principle And Interpretation

Ethyl Violet Azide Broth is based on the formulation of Litsky et al (3) and 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 (1).

Ethyl Violet Azide Dextrose Agar is a modification of Ethyl Violet Azide Broth (M426) (3) where 1.5% agar is added as a solidifying agent. It is used for detection and confirmation of Streptococci. It is based on original formulation of Litsky et al (4). Ethyl Violet Azide Dextrose Agar medium has 0.5% dextrose and was found equally productive as the medium described originally containing 1.5% dextrose. It was found that the medium with the lesser amount of carbohydrate was less adversely affected by heat during sterilization. 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).

E.V.A. Dextrose Agar contain 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.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.08% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M1397: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours

Organism	Inoculum (CFU)	Growth	Recovery
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	0%
<i>Enterococcus faecalis</i> ATCC 50-100 29212		good-luxuriant	>50%

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry date on the label.

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.

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