

TM 103 – ETHYL VIOLET AZIDE BROTH (E.V.A BROTH)

INTENDED USE

For selective and confirmatory detection of Enterococci as an indicator of faecal pollution in water.

PRODUCT SUMMARY AND EXPLANATION

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 bacterium in frozen foods.

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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

The medium consists of 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.

INSTRUCTION FOR USE

- Dissolve 35.80 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 psi 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 SPECIFICATIONS



Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light amber coloured, clear solution in tubes.
pH (at 25°C) : 7.0 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
<i>Escherichia coli</i>	25922	$\geq 10^3$	Inhibited	35-37°C	24-48 Hours
<i>Streptococcus pyogenes</i>	19615	50-100	Good-luxuriant with purple button at the bottom of tube	35-37°C	24-48 Hours
<i>Enterococcus faecalis</i>	29212	$\geq 10^3$	Inhibited	35-37°C	24-48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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



 GMP Good Manufacturing Practices Certified	 Best Before	 Quantity	 Catalogue Number	 Manufacturer
 Temperature Unit	 Lot / Batch Number	 Consults Instructions for Use	 QR Code	

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
*For Lab Use Only
Revision: 08 Nov., 2019