



PRODUCT SPECIFICATION SHEET

Ethyl Violet Azide Broth (E.V.A. Broth) (DM413)

Intended Use

Ethyl Violet Azide Broth (E.V.A. Broth) (DM413) is recommended for selective, confirmatory detection of *Enterococci* as an indicator of faecal pollution in water and other specimens.

Product Summary and Explanation

The presence of *Enterococci* acts as a valuable index of faecal or sewage pollution in water.⁽¹⁾ Mallmann and Seligmann⁽²⁾ compared various enrichment media for detecting fecal streptococci and found that Azide Dextrose Broth presumptively identified the streptococci. However, because gram-positive bacteria other than *Enterococci* grow in that medium, confirmation is necessary. 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⁽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. The medium known as Ethyl Violet Azide (EVA) Broth is specific for enterococci. In conjunction with Azide Dextrose Broth, EVA Broth is used to confirm the presence of enterococci. Ethyl Violet Azide Broth is based on the formulation of Litsky et al⁽⁴⁾ and the present medium is a modification of medium developed by Litsky et al⁽⁵⁾ with reduced amount of dextrose and increased dye concentration, making the medium highly specific for Enterococci. E.V.A. Broth is used in conjunction with Azide Dextrose Broth.

Principles of the Procedure

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 / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
Dextrose	5.00
Dipotassium phosphate	2.70
Monopotassium phosphate	2.70
Sodium chloride	5.00
Sodium azide	0.40
Ethyl violet	0.00083
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.





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3. 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.

Directions

1. Suspend 35.8 grams of the medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense in tubes in 10 ml amounts.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured, clear solution in tubes
Reaction of 3.58% solution	pH 7.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Escherichia coli</i> ATCC 25922	>=10 ³	inhibited
2.	<i>Enterococcus faecalis</i> ATCC 29212	50-100	good-luxuriant with purple button at the bottom of tube
3.	<i>Streptococcus pyogenes</i> ATCC 19615	>=10 ³	inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer appropriate references for standard test procedures.

Results

Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.





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Packaging

Product Name : Ethyl Violet Azide Broth (E.V.A. Broth)

Product Code : DM413

Available Pack sizes : 100gm/500gm

References

1. Litsky W., Mallmann W.L. and Fifield C.W., 1953, Am. J. Publ. Health, 43:873.
2. Mallmann and Seligmann. 1950. Am. J. Pub. Health 40:286.
3. Larkin, Litsky and Fuller, 1955, Appl. Microbiol., 3:98, 102, 104, 107.
4. Litsky W., Mallmann W.L. and Fifield C.W., 1955, Am. J. Publ. Health, 45:104.
5. 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.

Further Information

For further information please contact your local MICROMASTER Representative.



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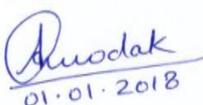
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