ETHYL VIOLET AZIDE (EVA) BROTH – LITSKY

Dehydrated and ready to use culture medium

Ethyl Violet Azide Broth; from left: uninoculated tube and tube with *Enterococcus faecalis*.

1 - INTENDED USE

Selective medium for the detection of enterococci in water and other samples.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20.00 g
Sodium chloride	5.00 g
Glucose	5.00 g
Dipotassium hydrogen phosphate	2.70 g
Potassium dihydrogen phosphate	2.70 g
Sodium azide	0.40 g
Ethyl violet	0.83 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterococci are considered a better indicator of sewage contamination than *Escherichia coli* as they are more resistant to chlorine.
Litsky et al.[1,2] developed a selective medium containing ethyl violet and sodium azide for the specific growth of enterococci from pure cultures or from glucose azide broth that showed growth when inoculated with sewage-contaminated water. They also devised a new test for enterococci, wherein glucose azide broth was used as a presumptive medium and ethyl violet azide broth as a confirmatory medium.
EVA Broth has been proposed, in conjunction with Azide Dextrose Broth, for enumeration of enterococci by MPN technique[3-6].
A similar procedure is included in the APAT, IRSA-CNR guidelines for the detection of faecal streptococci/enterococci in water using the MPN method.[6]
Tryptose provides nitrogen, amino acids and trace elements for microbial growth; sodium azide limits the growth of Gram-negative bacteria by blocking the enzyme cytochrome oxidase, and ethyl violet inhibits Gram-positive bacilli and Gram-positive cocci except enterococci. Glucose is a fermentable carbohydrate and a source of carbon and energy; phosphates are used as buffering agents to control the pH in the medium and sodium chloride contributes to maintaining the osmotic balance.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.8 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into tubes and sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Prepared tubes appearance	amber, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Ethyl Violet Azide (EVA) Broth - Litsky	Dehydrated medium	4014852	500 g (14 L)
Ethyl Violet Azide Broth	Ready-to-use tubes	551485	20 x 10 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, ancillary culture media and reagents.

8 – SPECIMENS

Water, food, milk, animal feeding stuffs, environmental samples in the area of food production and food handling, and other samples. Refer to applicable international standards and regulations for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

For the analysis, it is necessary to determine the volume according to the type and quality of water to be examined. For waste water or water of low quality, it is generally necessary to analysed decimal dilutions of the sample, whereas for treated water smaller dilutions and in any case different aliquots can be analysed.

1. Inoculate a series of tubes of Azide Dextrose Broth (REF 401105-551105) with appropriate graduated quantities of a 100 mL sample. Use sample volumes of 10 mL or less. The strength of the broth will be proportional to the sample size.
2. Incubate at 35-37°C for 24 ± 2 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
3. Remove 1 mL of broth culture from the positive tubes and inoculate into the corresponding tubes containing Ethyl Violet Azide Broth for confirmation testing. Incubate the tubes at 35-37 °C for 24+24 (±3) hours.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

10 - READING AND INTERPRETATION

Consider tubes with turbidity accompanied by a violet-grey deposit at the bottom of the tube as positive for enterococci.
After confirmation tests, apply MPN tables for estimating the number of enterococci per volumetric unit of sample.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 19433	37°C /48H-A	good growth with violet-grey deposit at the bottom of the tube
E. faecium ATCC 19434	37°C /48H-A	good growth with violet-grey deposit at the bottom of the tube
E. coli ATCC 25922	37°C /48H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Ethyl Violet Azide Broth (TB: Test Batch) is assessed for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 48 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}).
Productivity is tested with the following target strains: *E. faecalis* ATCC 29212*, E. faecalis* ATCC 19433, *E. faecium* ATCC 19434, *E. hirae* ATCC 10541. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1 and the tubes exhibit growth with a violet-grey deposit at the bottom of the tube.
Selectivity is tested with the following non-target strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *B. cereus* ATCC 11778. After incubation at 37°C for 48 hours, the growth of non-target strains is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- The MPN method with Azide Dextrose Broth/Ethyl Violet Azide Broth is not applicable to salt water as specified by APHA, for which the membrane filter technique is recommended.
- Since some Gram-positive bacilli and cocci other than faecal streptococci grow in Azide Dextrose Broth, a confirmation test in Ethyl Violet Azide Broth or other suitable medium is required.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Dehydrated Ethyl Violet Azide (EVA) Broth is classified as hazardous due to the presence of sodium azide. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.
- Each ready-to-use tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to MacFaddin, the prepared Ethyl Violet Azide Broth can be stored in the refrigerator for up to 6-8 weeks in screw cap tubes.[3]

Ready to use tubes

Upon receipt, store tubes in their original pack at +2°C / +8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date.

Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, atypical colour).

16- REFERENCES

1. Litsky W, Mallmann WL, Fifield CW. A new medium for the detection of enterococci in water. Am. J Pub Health 1953; 43:873
2. Litsky W, Mallmann WL, Fifield CW. Comparison of the most probable number of Escherichia coli and enterococci in rivers waters. Am J Public Health 1955;45.1049.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
4. APHA Standard Methods for the Examination of Water and Wastewater 14th ed. Washington, DC: American Public Health Association, 1975
5. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D.C. 1976
6. WHO Examination of water for pollution control. Part III: Biological, Bacteriological and Virological Examination., ed. Oxford. Pergamon Press, World Health Organization.1982
7. APAT, IRSA-CNR Manuali e Linee Guida 29/2003 Metodi analitici per le acque. Cap 3, 7040

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/07

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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