TECHNICAL DATA SHEET

ETHYL VIOLET AZIDE (LITSKY) BROTH

CONFIRMATION OF ENTEROCOCCI

1 INTENDED USE

Ethyl Violet Azide Broth (also known as Litsky broth) is used to carry out the confirmation test of the detection and enumeration of fecal streptococci (enterococci) in drinking water and waste water, in frozen foods and other food products by the most probable number method. The procedure involves two stages :

- presumptive test in Azide Dextrose Broth (Rothe)
- confirmation in Ethyl Violet Azide Broth (Litsky)

2 HISTORY

Ethyl Violet Azide Broth was formulated according to the recommendations of Litsky, Malmann and Fifield, who investigated the action of a number of dyes and selective agents for the formulation of a confirmation medium for fecal streptococci. The authors subsequently modified the original formula by reducing the glucose concentrations and increasing that of ethyl violet. The results indicated that the medium obtained was sufficiently specific for enterococci and that the few strains of sporulated bacilli and Gram-positive cocci other than fecal streptococci which gave false positives on Azide Dextrose Broth were inhibited by ethyl violet.

3 PRINCIPLES

Polypeptone and glucose supply the nutrient elements required for the development of enterococci.

The selectivity of the medium for enterococci is due to the presence of ethyl violet and sodium azide, which inhibit the growth of Gram-negative bacilli and sporulated Gram-positive species.

Phosphates buffer the medium and maintain the pH constant.

Sodium chloride maintains the osmotic balance of the medium.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Polypeptone ..... 20,0 g
- Glucose ..... 5,0 g
- Sodium chloride ..... 5,0 g
- Monopotassium phosphate ..... 2,7 g
- Dipotassium phosphate..... 2,7 g
- Sodium azide ..... 0,3 g
- Ethyl violet ..... 0,5 mg

pH of the ready-to-use media at 25 °C : 6,8 ± 0,2.

5 PREPARATION

- Dissolve 35,7 g of dehydrated media (BK061) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense into tubes at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**
35,7 g/L

✓ **Sterilization :**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

- Transfer a loop of culture from Azide Dextrose broth (Rothe) to a tube of Ethyl Violet Azide (Litsky) broth.
- Incubate at 37 ± 1 °C for 24 and 48 hours.

✓ **Inoculation :**
A loop from Rothe broth

✓ **Incubation :**
24 h and 48 h at 37 °C

7 RESULTS

The appearance of slight cloudiness and/or the formation of a violet deposit at the bottom of the tube indicate the presence of fecal streptococci.
NOTE : When the violet pellet deposited, the cloudiness of the medium may become very slight.

8 QUALITY CONTROL

Dehydrated media : beige powder, free-flowing and homogeneous.
Prepared media : amber solution, limpid.

Typical culture response after 48 hours of incubation at 37 °C

Microorganisms		Growth
(1) *Enterococcus faecalis*	WDCM 00087	Positive, violet deposit
(1) *Enterococcus faecalis*	WDCM 00176	Positive, violet deposit
Staphylococcus aureus	WDCM 00034	Inhibited
Bacillus subtilis	WDCM 00003	Inhibited

(1) Inoculum $\leq 10^2$ microorganisms.

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in tubes (*) : 180 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK061HA

11 BIBLIOGRAPHY

Litsky, W., Mallmann, W.L., and Fifield, C.W. 1953. A new medium for the detection of enterococci in water. Am. J. Public Health, 43 (7): 873.

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Rodier, J. 1984. L'analyse de l'eau. Dénombrement des streptocoques fécaux présumés (Méthode par ensemencement en milieux liquides). Dunod 7ème Ed., 825-828.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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