TECHNICAL DATA SHEET

AZIDE DEXTROSE BROTH (ACC. TO ROTHE)

DETECTION AND ENUMERATION OF ENTEROCOCCI

1 INTENDED USE

Azide Dextrose Broth (Rothe) is used for the enumeration of enterococci in drinking water, frozen foods and other food products by the most probable number method.

2 HISTORY

This sodium azide glucose broth is prepared according to the formula of Rothe. It was recommended by Malmann and Seligman for the enumeration of fecal streptococci in waste water and foods. Malmann, Botwright and Churchill showed the bacteriostatic action of sodium azide on Gram-negative flora, thereby favoring the growth of enterococci.

3 PRINCIPLES

The high nutritive capacity of Azide Dextrose Broth is due to the presence of a high concentration of polypeptone and glucose.

Sodium chloride maintains the osmotic equilibrium.

Potassium phosphates buffer the medium.

Sodium azide inhibits the growth of Gram-negative bacteria by its bacteriostatic action and favors that of fecal streptococci.

After observing cultures in tubes (presumptive test), it is necessary to carry out a confirmation in Ethyl violet Azide Broth (Litsky broth).

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 ..... 0,2 g

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

5 PREPARATION

Preparation of single strength media :

- Dissolve 35,6 g of dehydrated media (BK060) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense into tubes 16 x 160 mm, at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution : 35,6 g/L**

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

Preparation of double strength media :

- Dissolve 71,2 g of dehydrated media (BK060) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense into tubes 20 x 200 mm, at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**
71,2 g/L

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

6 INSTRUCTIONS FOR USE

- Transfer 10 mL of the sample to test to several tubes of broth at double concentration (according to the MPN method being used).
- Transfer 1 mL of the sample to test and its serial dilutions in to the tubes of single strength media as needed in the chosen protocol.
- Incubate all tubes at 37 ± 1 °C for 24 and 48 hours.

✓ **Inoculation :**
10 mL double strength
1 mL single strength

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

7 RESULTS

Positive tubes demonstrate turbidity.
Re-inoculate positive tubes into Litsky broth (BK061).

8 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.

Prepared media : amber solution, limpid.

Typical culture response after 24 hours of incubation at 37 °C, followed by subculturing in Litsky broth :

Microorganisms		Growth
(1) *Enterococcus faecalis*	ATCC® 33186	
+ *Escherichia coli*	WDCM 00013	Positive
+ *Pseudomonas aeruginosa*	WDCM 00024	
(1) *Enterococcus faecalis*	WDCM 00176	
+ *Salmonella* Enteritidis	WDCM 00030	Positive
+ *Staphylococcus aureus*	WDCM 00034	
Escherichia coli	WDCM 00013	Inhibited
Bacillus subtilis	WDCM 00003	Inhibited

(1) inoculum <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 BK060HA

11 BIBLIOGRAPHY

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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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