Rothe Broth (Glucose Broth With Azide)

Cat. 1238

For the quantitative determination of fecal enterococci

Practical information

Aplications	Categories
Selective enumeration	Enterococci

Principles and uses

Rothe Broth (Glucose Broth With Azide) is a selective medium recommended by Malmann and Seligmann for the quantification of enterococci in water, food and other materials suspect of being contaminated by waste waters. Enterococci are the best indicators of fecal contamination in water as Escherichia coli is very resistant to chloride.

The presence of enterococci is an indicator for fecal contamination, especially when it occurred a long time ago and the less resistant coliform bacteria, including Escherichia coli, may already be dead when the analysis is carried out.

Peptone mixture and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The use of Sodium azide to selectively inhibit Gram-negative bacteria first appeared in the studies of EDWARDS (1938) on the isolation of Streptococcus agalactiae, it was later showed that Sodium azide can also be used for the isolation of enterococci from water.

Formula in g/L

Glucose	7,5	Beef extract	4,5
Peptone mixture	15	Sodium azide	0,2
Sodium chloride	7,5		

Preparation

Suspend 34.7 grams of the medium in one liter of distilled water (69.4 grams if double concentration is desired). Mix well and dissolve by heating with frequent agitation until boiling point. DO NOT OVERHEAT. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes.

Instructions for use

Rothe Broth is ideal for the enumeration of enterococci by the serial dilution method. Inoculate 10 ml of the sample in 10 ml tubes of double-strength Rothe Broth (or 1 ml of the sample in 10 ml of a single - strength medium). Use 5 tubes for each dilution (according to Mallmann and Seligmann).

Incubate all tubes at 35 ± 2°C for 24 – 48 hours. Confirmation of fecal enterococci is obtained by the subsequent inoculation of positive tubes into EVA Broth (Cat. 1230).

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Yellowish brown	7,2 ± 0,2

Microbiological test

Incubation conditions: (35±2 ºC / 24-48 h)

Microrganisms	Specification
Enterococcus faecalis ATCC 19433	Good growth

Escherichia coli ATCC 25922	Inhibited growth
Staphylococcus aureus ATCC 25923	Inhibited growth
Enterococcus faecalis ATCC 29212	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Mallmann W.L. Seligmann E.B. AJPH, 1950. 40 286-289 Standard Methods for the Examination of Water and Wastewater. Eleventh Edition APHA Inc. New-York 1960
Edwards S.J. (1933) J. Comp. Path Therap., 46.211.