

Specification

Solid medium for the confirmation and enumeration of enterococci in clinical samples and water samples by the membrane filtration method according to ISO.

Formula * in g/L

Tryptone.....	17,00
Peptone.....	3,00
Yeast extract.....	5,00
Bile.....	10,00
Sodium chloride.....	5,00
Esculin.....	1,00
Ammonium ferric citrate.....	0,50
Sodium azide.....	0,15
Agar.....	15,00

Final pH 7,1 ±0,1 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 56.6 g of powder in 1 L of distilled water and bring to the boil. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Bile Esculin Azide Medium is a modification of the classical Bile Esculin proposed by Isenberg, Goldberg and Sampson in 1970, but with a reduction in the amount of bile and the addition of sodium azide. Brodsky and Schieman showed that this medium, also known as Pfizer Enterococci Selective Medium gave the best results using the membrane filtration technique.

The actual formulation according to the ISO Standard 7899-2:2000 is used for the second step in the confirmation and enumeration of enterococci in water by the membrane filtration method. The colonies previously selected in the Slanetz Bartley Agar must be confirmed by a short incubation on Bile Esculin Azide Medium for verification of esculin hydrolysis in a selective environment.

Technique

After an incubation of 24-48 hours on Slanetz Bartley Agar, the membrane filter showing typical colonies is transferred, with sterile forceps in an upright position, to a pre-warmed plate of Bile Esculin Azide Agar. After two hours of incubation at 44 ± 0.5°C the membrane filter is inspected. All the typical colonies that show brown to black colour in the surrounding medium are considered positive and therefore intestinal enterococci.

A heterogeneous distribution of the colonies or the presence of abundant and different microorganisms can interfere with the differentiation of positive colonies.

Quality control

Incubation temperature: 36°C ±2.0

Incubation time: 24 h

Inoculum: Practical range 100±20 CFU. Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity) / 10³-10⁴CFU (Specificity) according to ISO 11133:2014.

Microorganism
Growth
Remarks

<i>Escherichia coli</i> ATCC® 25922	Inhibited	Selectivity
<i>Enterococcus faecalis</i> ATCC® 29212	Productivity > 0.70	Black medium. E (+)
<i>Enterococcus faecalis</i> ATCC® 19433	Productivity > 0.70	Black medium. E (+)
<i>Staphylococcus aureus</i> ATCC® 25923	Inhibited	Selectivity
<i>Enterococcus faecalis</i> ATCC® 19433	Specificity - conform	Esculin (+) into MF. 44±0,5 ° C / 2h

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).