Bile Esculin Azide Agar ISO

Cat. 1005

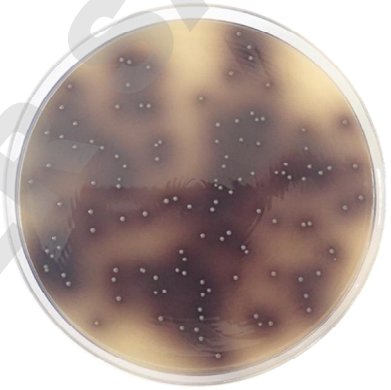
For the selective isolation and presumptive identification of intestinal enterococci by membrane filtration method

Practical information

Aplications	Categories
Selective isolation	Enterococci

Industry: Water

Regulations: ISO 7899-2



Principles and uses

Bile Esculin Azide Agar is a modification of Bile Esculin Agar (Cat. 1031), with the addition of sodium azide as an inhibitor and with the reduction of the bile concentration. The resulting medium is more selective but still provides rapid growth and efficient recovery of enterococci. The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci. Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the Ferric amonium citrate to form a dark brown or black colony. Ox bile does not inhibit enterococci while other Gram positive bacteria are inhibited. Sodium azide inhibits Gram negative bacteria. Tryptone, peptone and yeast extract supply the nutrients essential for growth. Sodium chloride provides the osmotic balance. Bacteriological agar is the solidifying agent.

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

Tolerance to bile and the ability to hydrolyze esculin constitutes a reliable presumptive test for the identification of enterococci. Appears a brown color (positive reaction) around the colonies.

Formula in g/L

Bacteriological agar	15	Esculin	1
Ferric ammonium citrate	0,5	Ox Bile	10
Peptone	3	Sodium azide	0,15
Sodium chloride	5	Tryptone	17
Yeast extract	5		

Preparation

Suspend 56,6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121ºC for 15 minutes. Cool to 50-60 ºC and dispense into appropriate containers. Overheating can cause darkening of the medium. If tubes are used, allow cooling in a slanted position.

Instructions for use

For the detection and enumeration of enterococci according to ISO 7899-2:

- Filter a measured volume of water through a membrane filter.
- Place the membrane on a Slanetz-Bartley Medium (Cat. 1109).
- Incubate at 36±2 ºC for 44±4 h.
- Transfer the membrane with characteristic colonies previously incubated in the Slanetz-Bartley medium (Cat. 1109), without inverting the membrane, to

a plate with Bile Esculin Azide Agar, pre-heated to 44ºC.
- Incubate at 44±0,5 ºC for 2 hours.
- Read the plate immediately.
- It is considered that the typical colonies that show a brown-black color in the surrounding medium give positive reactions and are recounted as intestinal enterococci.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Toasted	Litmus	7,1 ± 0,1

Microbiological test

Incubation conditions: (36±2 °C / 44±4 h).
Inoculation conditions: Productivity quantitative (100±20.Min.50 CFU) / Selectivity (10^4-10^6 CFU).
Reference media: TSA.

Microrganisms	Specification	Characteristic reaction
Streptococcus pyogenes ATCC 12344	Total inhibition	
Enterococcus faecalis ATCC 19433	Good growth	Brown-black color in the medium
Escherichia coli ATCC 25922	Total inhibition	
Enterococcus faecalis ATCC 29212	Good growth	Brown-black color in the medium
Enterococcus faecium ATCC 6057	Good growth	Brown-black color in the medium

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

ISO 7899-2. Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method.
Facklam, R.R. and M.D. Moody 1970. Presumptive identification of Group D Streptococci: The bile-esculin test. Appl. Microbiol 20:245.
Ruoff, K.L. 1995 Streptococcus. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (eds), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.