

Specification

Solid medium for the confirmation and enumeration of enterococci in water by the membrane filtration method according to ISO 7899-2.

Presentation

20 Prepared plates
90 mm
with: 21 ± 2 ml

Packaging Details

1 box with 2 packs of 10 plates/pack. Single cellophane.

Shelf Life

3 months

Storage

2-14°C

Composition

Composition (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

Description /Technique

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 (Art. No. 01 -579 + 06-023) 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.

After incubation, enumerate all the colonies that have appeared onto the surface of the agar. Typical colonies of *Enterococcus* sp. show a brown to black coloured halo.

Each laboratory must evaluate the results according to their specifications. Presumptive isolation of *Enterococcus* must be confirmed with further microbiological and biochemical tests.

Quality control**Physical/Chemical control**

Color : yellow

pH: 7.1 ± 0.1 at 25°C

Microbiological control

Incubate, MF w. microorganisms in SB at 37°C during 24-48 h and transfer in BEA medium.

Aerobic. Incubation at 44 °C, for 2h . Esculine Test.

Microorganism*Enterococcus faecalis* ATCC® 19433, WDCM 00009*Escherichia coli* ATCC® 25922, WDCM 00013*Enterococcus faecalis* ATCC® 29212, WDCM 00087*Enterococcus faecium* ATCC® 6057, WDCM 00177**Growth**

Good - Esculin Positive reaction

Inhibited

Good - Esculin Positive reaction

Good - Esculin Positive reaction

Sterility Control

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH

Check at 7 days after incubation in same conditions

Bibliography

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla.
- BRODSKY M.H. & D.A. SCHIEMANN (1976) Evaluation of Pfizer Selective Enterococcus and KF media for recovery of fecal streptococci from water by membrane filtration. Appl. Environ. Microbiol. 31 :695-699.
- ISENBERG, H.D., D. GOLDBERG & J. SAMPSON (1970) Laboratory studies with a Selective Enterococcus Medium. Appl. Microbiol. 20:433.
- ISO Standard 7899-2 (2000) Water Quality. Detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method.