ISO

Bile Aesuclin Azide Agar

For the detection and enumeration of intestinal enterococci (faecal streptococci) acc. to ISO 7899-2

Mode of Action

The presence of intestinal enterococci, also termed faecal streptococci, serves as an indicator for faecal contamination, particularly when the contamination took place a long time ago and the less resistant coliform bacteria, including Escherichia coli, may be already dead when the analysis is carried out.

Bile salt aesculin azide agar is employed acc. to ISO 7899-2 as a confirmation and enumeration medium for typical isolate on the primary isolation Membrane Enterococcus Selective Agar acc. to Slanetz and Bartley (Cat. no. 1.05262.0500 or 1.05289.0500).

Enterococci and some species of the genus Streptococcus namely S. bovis and S. equines can reproduce normally in this medium.

Esculin hydrolysis and bile tolerances are regarded as reliable characteristics of enterococci (FACKLAM 1971, 1973).

Intestinal Enterococci hydrolyse the glycoside esculin to give dextrose and esculetin. Esculetin forms an olive green to black complex with iron(III) ions.

Enterococci are bile tolerant. Bile salts inhibit the growth of numerous accompanying bacteria. The concentration of sodium azide present in this medium largely inhibits the growth of the accompanying Gram-negative microbial flora, while sparing the enterococci.

The use of sodium azide as a selective inhibitor for Gram-negative bacteria was reported in the studies of EDWARDS (1933, 1938) and HARTMANN (1936) on the isolation of Str. agalactiae. MALLMANN (1940) and SNYDER and LICHSTEIN (1940) later showed that sodium azide can also be used for the isolation of enterococci from water.

Typical Composition (g/litre)

Peptone from Casein 17.0; peptone 3.0; yeast extract 5.0; sodium chloride 5.0; aesculin 1.0; ammonium iron(III) citrate 0.5; ox bile 10.0; sodium azide 0.15; agar-agar 13.0:

Preparation

Suspend 54.65 g in 1 litre water and dissolve by boiling. Sterilise for 15 min. at 121 °C. After cooling to 45-50 °C pour into Petridishes to a depth of 3 mm to 5 mm and allow to solidify.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and yellow.

Storage

Poured plates can be stored at +2 - +8 °C for up to 2 weeks.

Experimental Procedure and Evaluation

For the confirmation typical red, maroon or pink coloured colonies on membrane filter Enterococcus selective agar acc. to Slanetz and Bartley (Cat. no. 1.05262.0500 or 1.05289.0500) are transferred, with sterile forceps without inverting the filter onto a plate of bile salt aesculin azide agar which has been pre-heated at 44 °C. After the inoculation plates are incubated at 44 ± 0.5 °C for 2h.

Regard all typical colonies showing a tan to black colouration in the surrounding medium as giving a positive reaction and count as intestinal enterococci.

Literature

ISO INTERNATIONAL STANDARDISATION ORGANISATION WATER QUALITY DETECTION AND ENUMERATION OF INTESTINAL ENTEROCOCCI PART 2 MEMBRANE FILTRATION **ISO 7899**-2 2000.

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Ordering Information

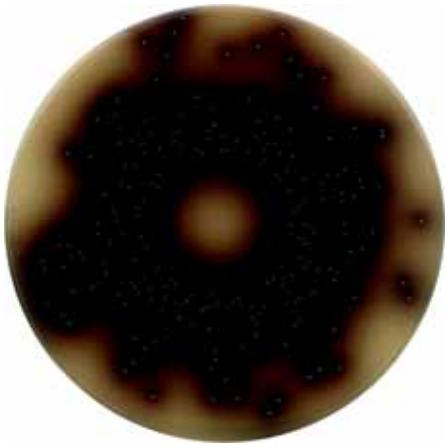
Product	Ordering No.	Pack size
Bile Aesuclin Azide Agar	1.00072.0500	500 g

Bile Aesuclin Azide Agar

Quality control

Test strains	Recovery rate (%)	Colony colour
Enterococcus faecium ATCC 882	≥ 60	Black
Enterococcus faecalis ATCC 19433	≥ 70	Black
Enterococcus durans ATCC 6056	≥ 50	Black
Enterococcus hirae ATCC 8043	≥ 60	Black
Listeria monocytogenes ATCC 19118	≤ 0.01	Colourless
Staphylococcus aureus ATCC 25923	≤ 0.01	Colourless
Escherichia coli ATCC 25922	≤ 0.01	Colourless

Enterococcus faecalis ATCC 19433



Enterococcus hirae ATCC 8043