



Bile Aesculin Azide Agar

For the detection and enumeration of intestinal
enterococci in water acc. to ISO 7899-2

Granulated Culture Media – Safer from Merck.



Bile Aesculin Azide Agar



Cat. No. 1.00072.0500
(500 g)

For the detection and enumeration
of intestinal enterococci (faecal streptococci)
in water 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 isolates 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. equinus* 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, but not 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 demin. water and dissolve by boiling. Sterilise for 15 min. at 121°C. After cooling to 45-50°C pour into Petri dishes 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. Poured plates can be stored at 2-8°C for up to 2 weeks.

Experimental procedure and evaluation

For confirmation typical red, maroon or pink coloured colonies on membrane filter Enterococcus selective agar acc. to SLANETZ and BARTLEY (Cat. No. 1.05262.050 or 1.05289.0500) are transferred with sterile forceps without inverting the filter onto a plate of Bile 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.

Further information about the advantages of Merck's Granulated Culture Media you will find in the following promotion materials:

- Granulated Culture Media (W.28611.2)
- TSE - "low risk" products (W.28612.0)



1



2



3



1 *Enterococcus faecalis* ATCC 19433
tan/black colored colonies
Bile Aesculin Azide Agar

2 *Enterococcus faecalis* ATCC 19433
tan/black colored colonies
Bile Aesculin Azide Agar

3 *Enterococcus faecalis* ATCC 19433
red/maroon/pink colored colonies
m-Enterococcus Selective Agar acc.
to SLANETZ and BARTLEY

Literature

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