TECHNICAL DATA SHEET

BEA AGAR (BILE, ESCULIN & AZIDE)

CONFIRMATION OF ENTEROCOCCI

1 INTENDED USE

Bile Esculin Azide Agar (BEA agar) is a selective medium used to isolate and enumerate enterococci in food and pharmaceutical products. It is also used for the enumeration of enterococci animal feed.

The typical composition responds to that defined in the mandatory application directives NF EN ISO 7899-2 and NF T90-421, for the confirmation of enterococci in water and in swimming pool water.

2 HISTORY

Rochaix first showed the interest of esculin hydrolysis for the identification of enterococci. Meyer and Schoefeld then showed that this hydrolysis in medium containing bile was an excellent test for the detection of group D streptococci. The first formulations described by Swan were subsequently modified by Isenberg, who obtained a medium which was both more selective and at the same time favored the rapid growth and good recovery of the bacteria sought.
The current formula is described in standards as a confirmation media for intestinal enterococci in water, when the enumeration is carried out by membrane filtration.

3 PRINCIPLES

Sodium azide inhibits contaminating Gram-negative bacteria.

Bacteriological bile inhibits the growth of Gram-positive microorganisms.

Enterococci hydrolyze esculin to glucose and esculetin. The esculetin produced forms a black complex in the presence of ferric ions arising from ferric citrate in the medium.

4 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance..
For 1 liter of media :

- Tryptone ..... 17,00 g
- Peptic digest of meat..... 3,00 g
- Yeast extract ..... 5,00 g
- Bacteriological ox bile ..... 10,00 g
- Sodium chloride ..... 5,00 g
- Esculin..... 1,00 g
- Ferric ammonium citrate ..... 0,50 g
- Sodium azide ..... 0,15 g
- Bacteriological agar..... 13,00 g

pH of the ready-to-use medium at 25°C : 7,1 ± 0.1.

5 PREPARATION

Preparation of dehydrated media :

- Suspend 54,6 g of dehydrated medium (BK158) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring until complete dissolution.
- Dispense in tubes or flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47°C.
- Pour into sterile Petri dishes of the appropriate size.

✓ **Reconstitution : 54,6 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use of ready-to-melt media :

- Melt the medium (if it was prepared in advance) or the ready-to-melt (BM104) with the minimum amount of time necessary in order to achieve total liquefaction.

6 INSTRUCTIONS FOR USE

Confirmation of intestinal enterococci in water :

- After culturing on Slanetz & Bartley,agar, transfer the membrane without turning over (filtered side up) onto undried plates.
- Incubate at 44 ± 1 °C for 2 hours.

✓ **Inoculation : Membrane filter**

✓ **Incubation : 2 h at 44 °C**

Enumeration in animal feeds :

- Dry in an incubator with the covers partially removed.
- Inoculate on the surface of the medium with 0,1 mL of the sample to test and its serial dilutions.
- Spread the inoculum on the surface with the aid of a sterile triangle.
- Incubate at 37 ± 1 °C for 24 ± 2 hours.

✓ **Inoculation : 0,1 mL on surface**

✓ **Incubation : 24 h at 37 °C**

7 RESULTS

After growth on Slanetz & Bartley agar and transfer of the membrane onto BEA agar, the typical colonies demonstrate a brown to black coloration in the medium.
In direct enumeration on BEA agar, the enterococci appear as small translucent colonies surrounded by a black halo. *Staphylococci* and yeasts may yield opaque colonies without a black halo. It is indispensable to identify suspected bacteria, especially to eliminate confusion with *Listeria* which may give rise to colonies similar to those of enterococci.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : cream powder, free-flowing and homogeneous.
Prepared media : amber agar with bluish glints.
Typical culture response after 24 h incubation at 37 °C :

Microorganisms		Growth (Productivity Ratio : P_R)
Enterococcus faecalis	WDCM 00087	$P_R \geq 50$ %
Escherichia coli	WDCM 00013	Inhibited, score 0

Esculin hydrolysis after 2 hours at 44 °C, (FD T90-461) :

Microorganisms		Reaction in 2 hours
Enterococcus faecalis	WDCM 00176	Positive
Aerococcus viridans	WDCM 00061	Negative

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
Ready-to-melt media in vials : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared media in vials (*) : 180 days at 2-8 °C.
Prepared media in plates (*) : 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK158HA

Ready-to-melt media :
10 x 100 mL vials BM10408

11 BIBLIOGRAPHY

Isenberg, H.D., Goldberg, D. and Sampson, J.. 1970. Laboratory Studies with a Selective *Enterococcus* Medium. Applied Microbiology, **20 (3)** : 433-436.

Rodier, J. 1984. L'analyse de l'eau. Dénombrement des streptocoques fécaux présumés (Méthode par ensemencement en milieux liquides). Dunod 7ème Ed., 825-828.

NF EN ISO 7899-2. Août 2000. Qualité de l'eau. Recherche et dénombrement des entérocoques intestinaux. Partie 2 : Méthode par filtration sur membrane.

NF T 90-421. Août 2006. Essais des eaux. Examens bactériologiques des eaux de piscines.

NF EN 15788. Décembre 2009. Aliments des animaux. Isolement et dénombrement de l'entérocoque (*E. faecium*) spp.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : BEA_EN v10
Creation date : 06-2003
Updated : 06-2017
Origin of revision : Correction of pH variation

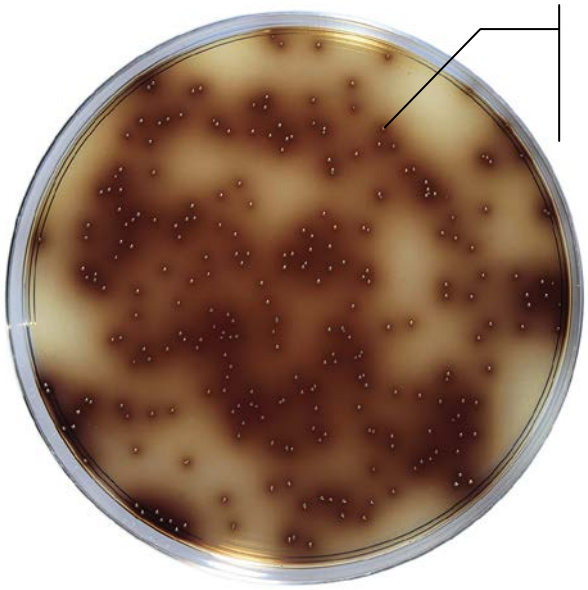
ANNEX 1 : PHOTO SUPPORT

BEA Agar

Detection and enumeration of *Enterococci*.

Results :

Growth obtained after 24 hours of incubation at 37 °C.



Enterococcus faecalis

Characteristic colonies :
Small, translucid colonies surrounded by a black halo .