TECHNICAL DATA SHEET

SLANETZ AND BARTLEY AGAR

ENUMERATION OF ENTEROCOCCI

1 INTENDED USE

Slanetz and Bartley Agar is a selective medium used for the enumeration of intestinal enterococci in drinking water, beverages, waste water, swimming pool water and various biological products of animal origin, by the membrane filtration method.

The typical composition responds to that defined in the standards NF EN ISO 7899-2 and NF T90-421.

2 HISTORY

The medium was formulated by Slanetz *et al*. in order to enumerate enterococci in water and beverages by the membrane filtration method. A modification of the method, involving the addition of TTC (triphenyltetrazolium chloride) leads to better counts when the membranes are placed directly on the surface of the agar. The current formula produces results comparable to those of the method developed by Litsky, Mallmann and Fifield for the detection of fecal streptococci..

3 PRINCIPLES

Sodium azide inhibits the growth of Gram-negative bacteria.

TTC is an indicator of bacterial growth. It is reduced to an insoluble formazan inside the cells. This reaction is seen by the formation of red to maroon colonies.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Tryptose ..... 20,0 g
- Yeast extract ..... 5,0 g
- Glucose ..... 2,0 g
- Dipotassium phosphate ..... 4,0 g
- Sodium azide ..... 0,4 g
- 2, 3, 5 triphenyltetrazolium chloride ..... 0,1 g
- Bacteriological agar ..... 10,0 g

pH if the ready-to-use media at 25 °C : 7,2 ± 0,2.

For 41,5 g of dehydrated media BK037

- Tryptose ..... 20,0 g
- Yeast extract ..... 5,0 g
- Glucose ..... 2,0 g
- Dipotassium phosphate ..... 4,0 g
- Sodium azide ..... 0,4 g
- 2, 3, 5 triphenyltetrazolium chloride ..... 0,1 g
- Bacteriological agar ..... 10,0 g

For 41,4 g of dehydrated media BK129

- Tryptose ..... 20,0 g
- Yeast extract ..... 5,0 g
- Glucose ..... 2,0 g
- Dipotassium phosphate ..... 4,0 g
- Sodium azide ..... 0,4 g
- Bacteriological agar ..... 10,0 g

For a vial of supplement BS027

- 2, 3, 5 triphenyltetrazolium chloride ..... 50 mg

5 PREPARATION

Preparation using complete dehydrated media :

- Dissolve 41,5 g of complete dehydrated media (BK037) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Avoid excessive heating.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates (the thickness of the agar should be at least 5 mm).
- Let solidify on a cold, flat surface.

✓ **Reconstitution :**
41,5 g/L

✓**Sterilization :**
Bring to a boil.

Preparation using dehydrated base media (without TTC) :

- Dissolve 41,4 g of dehydrated base media (BK129) in 1 liter of distilled or demineralized water .
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- If necessary, dispense into 100 mL vials and autoclaver for 20 minutes at 110 °C. Ordinarily, the media can be used without autoclaving.
- Cool and maintain the media in a molten state at 44-47 °C.
- Rehydrate a vial of TTC 50 mg supplement (BS027) with 5 mL sterile water.
- Aseptically add 1 mL of reconstituted TTC 50 mg supplement (BS027) per 100 mL volume of base.
- Mix well.
- Pour into sterile Petri plates (the thickness of the gel should be at least 5mm).
- Incubate at 36 ± 2 °C for 44 ± 4 hours.

✓ **Reconstitution :**
41,4 g/L

✓ **Sterilization:**
If necessary, 20 min at 110 °C

✓ **Inoculation :**
Membrane filtration

✓**Incubation :**
44 ± 4 h at 36 ± 2 °C

6 INSTRUCTIONS FOR USE

- Aseptically filter through a nitrocellulose membrane a set volume of water sample.
- To the surface of plates prepared as above or onto pre-poured media (BM094 or BM146), previously brought to room temperature, deposit the membrane onto the surface of the agar, filtered side up, insuring a proper and uniform contact with the agar surface.
- Incubate at 36 ± 2 °C for 44 ± 4 hours.

✓ **Inoculation :**
Membrane filtration

✓**Incubation :**
44 ± 4 h at 36 ± 2 °C

7 RESULTS

Red, maroon or pink colonies are considered as characteristic.
Carry out confirmation of typical colonies using Bile Esculin Azide Agar (BK158 or BM104), preheated to 44°C.

See ANNEX 1 : PHOTO SUPPORT .

8 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Lyophilisate appearance : white, giving rise to a colorless, limpid solution after reconstitution.
Prepared (complete) media : amber to pink-orange agar.

Typical culture response after 44 hours of incubation at 36 °C (NF EN ISO 7899-2, NF EN ISO 11133) :

Microorganisms		Growth
Enterococcus faecalis	WDCM 00009	$P_R \geq 50$ %
Enterococcus faecalis	WDCM 00176	$P_R \geq 50$ %
Enterococcus faecium	WDCM 00178	$P_R \geq 50$ %
Escherichia coli	WDCM 00013	Inhibited, score 0
Staphylococcus aureus	WDCM 00009	Inhibited, score 0
Staphylococcus aureus	WDCM 00034	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated (complete) media (BK037) : 2-30 °C, shielded from light.
Dehydrated base media without TTC (BK129) : 2-30 °C.
TTC 50 mg Supplement: 2-8 °C, shielded from light.
Pre-poured media in Petri plates : 2-8 °C, shielded from light.
The expiration dates are indicated on the labels.

Prepared base media without TTC in vials and autoclaved(*) : 180 days at 2-8 °C.
Prepared complete media in vials(*) : Not recommended.
Rehydrated supplement (*) : 30 days at 2-8 °C, shielded from light.
Prepared complete media in plates (*) : 30 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated complete media :
500 g bottle BK037HA

Dehydrated base media (without TTC) :
500 g bottle BK129HA
TTC 50 mg Supplement:
10 vials qsp 500 mL BS02708

Pre-poured complete media in Petri plates (Ø 55 mm) :
20 plates BM14608
120 plates BM09408

11 BIBLIOGRAPHY

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Rodier, J. 1984. L'analyse de l'eau. Dénombrement des streptocoques fécaux présumés. (Méthode par filtration sur membrane). Dunod 7è Ed., 828-829.

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 T90-421. Aout 2006. Qualité de l'eau - Examens bactériologiques des eaux de piscines.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau – préparation, stockage et essais de performance des milieux de culture – Microbiologie des aliments pour animaux et des eaux.

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.

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ANNEX 1 : PHOTO SUPPORT

Slanetz & Bartley Agar

Enumeration of *Enterococci*

Results :

Growth obtained after 44 hours of incubation at 36 °C (membrane filtration).

Enterococcus faecalis
Characteristic colony :
Red to maroon color
(Reduction of TTC)