TECHNICAL DATA SHEET

TRYPTONE-SOY AGAR (BASE)

BASE FOR BLOOD AGAR

1 INTENDED USE

Tryptone-Soy Agar, used as a base to be supplemented with blood, is prepared with selected starting materials which do not turn brown. It was specially designed to detect beta-hemolytic reactions and to favor the growth of particularly fastidious aerobic and anaerobic bacteria.
The media can be used for the hemolysis test on presumed colonies of *Bacillus cereus*, according to the standard ISO 21871.

2 PRINCIPLES

The combination of casein digest and papaic digest of soybean meal leads to optimal growth due to synergy between the protein supply of casein and the carbohydrate supply of soybeans.

Sodium chloride maintains osmotic balance.

Sterile defibrinated sheep blood used to enrich the medium demonstrates the hemolysis characteristics of different bacteria.

3 TYPICAL COMPOSITION

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

For 1 liter of media :

- Tryptone ..... 15,0 g
- Papaic digest of soybean meal ..... 5,0 g
- Sodium chloride ..... 5,0 g
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25 °C : 7,3 ± 0,2.

4 PREPARATION

- Dissolve 40,0 g of dehydrated media (BK028) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in vials of 100 mL.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Aseptically add 5 to 7 mL of defibrinated sterile sheep blood per vial.
- Mix well.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.

✓ **Reconstitution :**
40,0 g/L

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

NOTE :
For other applications, refer to the desired protocol in vigor.

5 INSTRUCTIONS FOR USE

Detection of hemolysis

- Inoculate in order to observe isolated colonies.
- Incubate at 30 °C for 24 hours for the growth of presumed *Bacillus cereus*, or from 24 to 48 h at 37 °C for the growth of other microorganisms.

CAMP Test

- Inoculate a 10 hour culture of beta-hemolytic *Staphylococcus aureus* ATCC® 33862 as a single median streak.
- Perpendicular to the initial streak, streak a culture of the streptococcus to identify, approaching the first as closely as possible (2-3 mm) without touching it it.

6 RESULTS

Beta Hemolysis

Group A hemolytic streptococci are small gray colonies, translucent or opaque, surrounded by a zone of beta type hemolysis. Other bacteria may exhibit the same type of hemolysis : *Listeria*, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.
Staphylococci give opaque colonies that are golden yellow or white, with or without β-type hemolysis zones.
Listeria present small zones of beta hemolysis.
Bacillus cereus present a clear zone around the colonies.

See ANNEX 1 : PHOTO SUPPORT.

Alpha hemolysis

Pneumococci appear as flat, smooth, grayish and sometimes mucous colonies, surrounded by a narrow greenish zone of alpha type hemolysis.

CAMP Factor

Group B streptococci produce a heat-resistant extracellular substance (CAMP factor) which leads to a triangle of total hemolysis in the zone of incomplete hemolysis of the staphylococcus, at the junction of the two cultures.

7 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Prepared media (with 5 % defibrinated sheet blood) : red, opaque agar.

Typical culture response after 48 hours of incubation at 37 °C in the presence of 5% sterile defibrinated sheep blood, qualitative method of inoculation :

Microorganisms		Growth
*Streptococcus pneumoni*ae	ATCC 6303	Good, score 2
Streptococcus pyogenes	ATCC 19615	Good, score 2
Listeria monocytogenes	WDCM 00020	Good, score 2
Listeria monocytogenes 4b	WDCM 00021	Good, score 2
Bacillus cereus	WDCM 00001	Good, score 2

8 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
The expiration date is indicated on the label.

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

9 PACKAGING

Dehydrated base media :
500 g bottle BK028HA

10 BIBLIOGRAPHY

Facklam, R.R., and Carey, R.B.. 1985. *Streptococci* and *Aerococci* in Lennette, E.H., Balows, A., Hausler, W.J., and Shadomy, H.J. (ed). Manual of Clinical Microbiology 4 th Ed., ASM Washington DC, 154-175.

McFaddin, J.F.. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilklins, Baltimore, volume 1: 794-806.

NF EN ISO 21871. Juillet 2006. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Bacillus cereus* présumés en petit nombre. Technique du nombre le plus probable et méthode de recherche.

11 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 : TRYPTONE SOY AGAR BASE_ENv8
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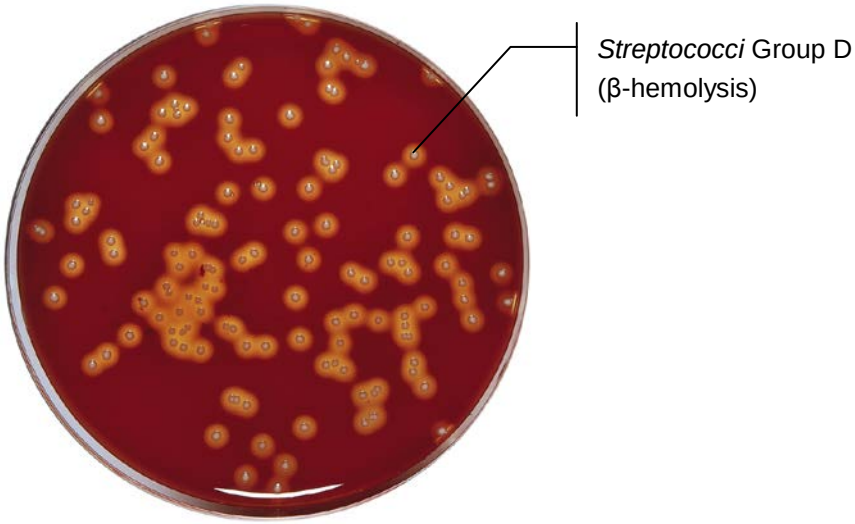
ANNEX 1 : PHOTO SUPPORT

Tryptone-soy agar (base for blood)

Blood agar

Results :

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



Characteristics : β-hemolysis : well defined and clear zones of clearing around colonies.
α-hemolysis : incomplete zones of clearing around the colonies with a greenish tint.