

Specification

Selective medium from isolation of gram-positive cocci of clinical samples.

Presentation

10 Prepared bottle
Bottle 125 ml
with: 90 ± 3 ml

Packaging Details

1 box with 10 bottles 125 ml. Non injectable cap (metallic).

Shelf Life

12 months

Storage

8-25°C

Composition

Composition (g/l):

Casein pancreatic digest..... 10.000

Meat peptic digest..... 5.000

Heart Pancreatic digest..... 3.000

Yeast Extract..... 5.000

Sodium chloride..... 5.000

Starch..... 1.000

Agar..... 15.000

Colistin sulphate..... 0.010

Nalidixic ac..... 0.015

Description /Technique

Description:

This medium is a modification of the Blood Columbia Agar with the selective antimicrobial agents Colistin sulfate and Nalidixic Acid added. These antibiotics inhibit the growth of Enterobacteriaceae and *Pseudomonas*.

Techniques recommended use:

The medium should be melted (100 °C) only once and used. Do not apply direct heat to melt it, nor be melted repeatedly. Cool to 45-50 °C. Aseptically add 5% defibrinated sterile sheep blood. Mix well before pouring.

Inoculate the samples directly on the surface of agar, streaking to obtain isolated colonies. Some stab inoculations should also be carried out to deposit Beta-haemolytic streptococci deep in the medium as this subsurface growth allows manifestation of both oxygen-stable and oxygen-labile streptolysin activity, giving clear haemolytic reactions.

The plates are incubated in (aerobic, anaerobic or 5-10% CO₂ enriched atmosphere) according to laboratory protocol, for each sample type. After incubation for 18 to 24 /48 hours at 35±2°C the plates are examined for growth and, subsequently, for haemolytic reactions:

- Alpha-haemolysis (a) is the reduction of haemoglobin to methaemoglobin in the medium surrounding the colony, producing a green halo.

- Beta-haemolysis (b) is the total lysis of the blood erythrocytes producing a clear zone around the colony.

- Gamma-haemolysis (g) is indicated by no haemolysis: No change in the environment.

- Alpha-prime-haemolysis (a) presents as a zone of complete lysis next to the colony surrounded by an area of partial lysis.

The haemolytic effect of streptococci depends on many factors. Ruoff (1995) noted that incubation in atmospheres enriched in (5 -10%) CO₂ optimized the action of beta-haemolytic streptococci and some strains of streptococci, (Lancefield group D) behave

differently depending on the animal origin of the blood used in the medium: In Blood Agar with horse, human, or rabbit blood, beta-haemolytic action is manifested and with sheep blood alpha-haemolytic action is best observed.

Different animal blood source, greater incubation times, humidity or larger percentage of carbon dioxide in atmosphere,... may be required depending on the sample, on the specifications of the laboratory, the expected isolations to be found.

Quality control**Physical/Chemical control**

Color : Brownish

pH: 7.2 ± 0.2 at 25°C

Microbiological controlSpiral Spreading: Practical range 100±20 CFU; Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity).

Previous addition of 5% Defibrinated Sheep Blood

Microaerophilic incubation at 35±2°C for 18-24h

Microorganism*Streptococcus pyogenes* ATCC® 19615*Stph. aureus* ATCC® 25923, WDCM 00034*Streptococcus pneumoniae* ATCC® 49619*Proteus mirabilis* ATCC® 12453**Growth**

Good (Beta-hemolysis)

Good (Beta-hemolysis)

Good (Alfa-hemolysis)

Inhibited

Sterility Control

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH

Check at 7 days after incubation in same conditions

Bibliography

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