

## Specification

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

## Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm with: 19 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14 °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
Defibrinated Sheep Blood.....	50 ml

## 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:

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% CO<sub>2</sub> enriched atmosphere) according to laboratory protocol, for each sample type. After incubation for 18 to 24 /48 hours at 37°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%) CO<sub>2</sub> 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 : Red

pH: 7.2 ± 0.2 at 25°C

### Microbiological control

Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10<sup>4</sup>-10<sup>6</sup> CFU (selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

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 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

## Bibliography

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- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- RUOFF, K.L. (1995) Streptococcus p. 299-305. En Manual of Clinical Microbiology 6<sup>th</sup> ed. Por Murray, Baron, Pfaller, Tenover y Yolkner (editors) ASM. Washington DC. USA.

## Storage

Storage conditions: 2-14°C

Avoid direct contact with surfaces that can freeze product.