Product :
COLUMBIA CNA AGAR BASE

Specification

Solid medium used, with the addition of blood for the selective isolation of Gram positive cocci, from clinical samples.

Formula * in g/L

Peptone mixture..... 20,000
Meat extract..... 3,000
Starch..... 1,000
Sodium chloride..... 5,000
Nalidixic acid sodium salt..... 0,015
Colistin sulfate..... 0,010
Agar..... 15,000

Final pH 7,3 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 44 g of powder in 950 mL of distilled water and heat to boiling point. Sterilize for 15 minutes at 121°C. Cool to 45-50° C and aseptically add defibrinated blood in a proportion of 5%. Mix gently and pour plates, avoiding the formation of bubbles.

Description

Columbia CNA Agar Base was first described in 1966 by Ellner *et al.* to selectively isolate Gram positive cocci and fungi in urine cultures, it was found that the addition of colistin and nalidixic acid significantly suppressed the growth of Gram negative bacteria. It contains a balanced mixture of peptones which, together with the meat extract, are a very good source of carbon, nitrogen and vitamins. The starch promotes the growth of *Neisseria* and the added blood enhances the haemolytic reaction of some streptococci. Sodium chloride maintains the osmotic balance and agar acts as a gelling agent. Blood is an additional source of growth factors and the basic constituent for the determination of haemolytic reactions.

Colistin solubilises the cell membrane of Gram negative bacteria and is especially effective against *Pseudomonas*. Nalidixic acid blocks DNA replication, especially in Enterobacteriaceae, and also in other Gram negatives. The combination of these two antibiotics is very effective in suppressing the growth of Enterobacteriaceae and members of the genus *Pseudomonas*, allowing the yeasts, staphylococci, streptococci and enterococci to grow more freely.

Some Gram negative bacteria such as Gardnerella vaginalis and Bacteroides species grow well in this environment, and some tiny colonies of *Proteus* may also grow. Some strains of streptococci, despite the nutritional richness of the environment, can grow poorly and may fail to grow.

Technique

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 hours at 35ºC 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.

Quality control

Incubation temperature: 35 °C±2,0 **Incubation time:** 21 ± 3 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 . MIcroAER

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC® 25923	Good	ß-hemolysis
Streptococcus pyogenes ATCC® 19615	Good	ß-hemolysis
Streptococcus pneumoniae ATCC® 49619	Good	á-hemolysis
Proteus mirabilis ATCC® 12453	Inhibited	-

References

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· RUOFF, K.L. (1995) Streptococcus p. 299-305. En Manual of Clinical Microbiology 6th ed. Por Murray, Baron, Pfaller, Tenover y Yolker (editors) ASM. Washington DC. USA.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).