ALLIANCE BIO EXPERTISE

Reference: FLWR3066 **A.B.E. - Technical Data Sheet**

Product: **Columbia CNA Agar**

Specification

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

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottle Bottle 125 ml with: 90 ± 3 ml	1 box with 10 bottles 125 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	12 months	8-25 ºC

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 ºC) only once and used. Do not apply direct heat to melt it, nor be melted repeatedly. Cool to 45-50° C. Aseptically add 5% desfibrinated 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% CO_2 enriched atmosphere) according to laboratory protocol, for each sample type. After incubation for 18 to 24 /48 hours at 35±2ºC 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_2 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.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

Quality control

Physical/Chemical control

Color : Brownish pH: 7.2 ± 0.2 at 25ºC

Microbiological control

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

Previous addition of 5% Defibrinated Sheep Blood

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

Microaerofilic incubation at 35 ± 2 ºC for 18-24h

Microorganism	Growth
Streptococcus pyogenes ATCC® 19615	Good (Beta-hemolysis)
Stph. aureus ATCC® 25923, WDCM 00034	Good (Beta-hemolysis)
Streptococcus pneumoniae ATCC® 49619	Good (Alfa-hemolysis)
Proteus mirabilis ATCC® 12453	Inhibited

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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