Columbia CNA Agar Base

Cat. 1152

Recommended for the isolation of Gram-positive cocci of clinical samples and other materials when used with blood

Practical information

Aplications	Categories
Selective isolation	Streptococcus

Industry: Clinical / Food

CE IVD

Principles and uses

Columbia CNA Agar Base is a modification of the Columbia Agar Base with the selective antimicrobial agents colistin sulfate and nalidixic acid added (CNA). These agents inhibit the growth of Enterobacteriaceae and Pseudomonas while they allow the growth of yeast, staphylococci, streptococci and pneumococci.

Colistin breaks the cell membrane of Gram-negative microorganisms, especially Pseudomonas species. The nalidixic acid blocks the DNA replication of susceptible bacteria and acts against many Gram-negative bacteria. The growth of most anaerobic bacteria is promoted by growth nutrients and stimulants such as nitrogen, vitamins, minerals and amino acids contained in the Peptone mixture and beef extract. Corn starch increases growth of Neisseria spp., and enhances the hemolytic reactions of some streptococci. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. Blood is an additional source providing the microorganisms with growth factors and is the basis for determining haemolytic reactions.

Hemolytic patterns may vary according to the blood or base medium types used. For example, defibrinated sheep blood allows the recovery of Thermophilus species and gives best results for group A streptococci.

Some Gram-negative microorganisms, like Gardnerella vaginalis, and some species of bacteria can grow in Columbia CNA Agar with added blood.

Formula in g/L

Bacteriological agar	15	Maize starch	1
Beef extract	3	Nalidixic acid	0,015
Peptone mixture	20	Sodium chloride	5
Colistin sulfate	0,01		

Preparation

Suspend 44 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. AVOID OVERHEATING. Cool to 45-50 °C and aseptically add 5-10% sterile defibrinated sheep blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood.

Instructions for use

For clinical diagnosis, the type of samples are
secretions of the respiratory tract.

- Inoculate the samples on the surface of the agar, streaking to obtain isolated colonies.
- Incubate at 35±2 °C for 18- 4 hours.
- Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5-10% CO2.
- Examine the hemolytic reactions.

Types of Hemolysis:
1. Alpha-hemolysis: greenish discoloration of medium.
2. Beta-hemolysis: clear zone around the colony.
3. Gamma-hemolysis: no change.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Clear amber. With blood: cherry red	7,3 ± 0,2

Microbiological test

Incubation conditions, with blood added: (35±2 ºC, CO2 atmosphere / 18-24 h)

Microorganisms	Specification	Characteristic reaction
Proteus miriabilis ATCC 12453	Inhibited growth	
Streptococcus pyogenes ATCC 19615	Good growth	Beta hemolysis
Staphylococcus aureus ATCC 25923	Good growth	Beta/Gamma hemolysis
Streptococcus pneumoniae ATCC 6305	Good growth	Alpha hemolysis

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Ellener, P.C., C.J. Stoessel, E. Drakeford, and F. Vassi 1966. A new culture medium for medical bacteriology. Am J. Clin Pathol. 45:502-504.
Ruoff, k., l. 1995 Streptococcus