COLUMBIA CNA AGAR

- For in vitro use only - Catalogue No. PC66

Our Columbia CNA Agar is a selective medium used in the isolation of gram-positive cocci from clinical and non-clinical materials.

Columbia Agar was developed by Ellner et al. in 1966 as a nutritious blood medium that gave good growth and sharply defined hemolysis reactions. Ellner and company also described a selective formulation with the addition of the antimicrobial agents, colistin and nalidixic acid. Colistin, a polypeptide antibiotic of the polymyxin group, and nalidixic acid, a first-generation quinolone, are primarily active against gram-negative bacteria thereby making Columbia CNA Agar a good medium for the selective isolation of gram-positive cocci. *Enterobacteriaceae* and *Pseudomonas* species are suppressed while allowing yeast, staphylococci, streptococci, and enterococci to grow.

Formula per Litre of Medium

Casein-Meat Peptone	10.0 g
Casein-Yeast Peptone	10.0 g
Heart Peptone	3.0 g
Sodium Chloride	5.0 g
Corn Starch	1.0 g
Agar	13.5 g
Defibrinated Sheep Blood	50.0 mL
Colistin Sulfate	10 mg
Nalidixic Acid	15 mg

pH 7.3 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature prior to inoculation.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. If desired, stabs into the medium can also be made to improve and better observe hemolytic reactions.
3. Depending on the organism of interest, incubate plates aerobically, anaerobically, or in a CO_2-enriched environment at 35°C.
4. Examine after 24 and 48 hours.

Interpretation of Results

After the incubation period, examine plates for growth and hemolysis. Hemolysis is a useful differential characteristic that is best viewed when a bright light is transmitted from behind the plate. Four different types of hemolysis can be described:

1. Alpha-hemolysis (α) – Partial hemolysis that results in a greenish discoloration around the colony
2. Beta-hemolysis (β) – Complete lysis of red blood cells resulting in a clear zone around the colony
3. Gamma-hemolysis (γ) – No hemolysis resulting in no change in the medium
4. Alpha-prime-hemolysis (α′) – A small zone of complete hydrolysis that is surrounded by an area of partial hemolysis

Additional biochemical or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *The incubation atmosphere has shown to influence hemolytic reactions of some streptococci; for optimal results incubate plates in a CO2-enriched environment or anaerobically*

Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Streptococcus pyogenes ATCC 19615	Growth, β-hemolysis
Streptococcus pneumoniae ATCC 6305	Growth, α-hemolysis
Staphylococcus aureus ATCC 25923	Growth
Proteus mirabilis ATCC 12453	Inhibition (partial)

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Storage and Shelf Life

Our Columbia CNA Agar should be protected from light and stored at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions the medium has an 8 week shelf life from the date of manufacture.

References

1. Ellner PD, Stoessel CJ. Drakeford E, Vasi F. A new culture medium for medical bacteriology. Am J Clin Pathol 1966; 45:502-4.
2. Goldberg RL, Washington JA, II. Comparison of isolation of *Haemophilus vaginalis* (*Corynebacterium vaginale*) from peptone-starch dextrose agar and Columbia colistin-nalidixic acid agar. J Clin Microbiol 1976; 4:245.
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
5. NCCLS, Quality assurance for commercially prepared culture media. 2nd ed; approved standard. NCCLS document M22-A2. Wayne, PA: NCCLS, 1996.