Columbia CNA Agar (NCM0115)

Intended Use
Columbia CNA Agar is used with blood for the selective isolation of Gram-positive cocci in a laboratory setting. Columbia CNA Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Ellner et al. described Columbia CNA Agar as a variation of Columbia Blood Agar Base that is selective for Gram-positive cocci. Colistin and Nalidixic Acid are added to the formula, selecting for Gram-positive organisms and fungi by suppressing Gram-negative bacteria.

Typical Formulation

Enzymatic Digest of Casein	5.0 g/L
Enzymatic Digest of Animal Tissue	8.0 g/L
Enriched Peptone	10.0 g/L
Starch	1.0 g/L
Sodium Chloride	5.0 g/L
Colistin	0.015 g/L
Nalidixic Acid	0.01 g/L
Agar	14.0 g/L

Final pH 7.3 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Suspend 43 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Do not overheat medium.
4. Cool to 45 - 50°C.
5. Prepare 5% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium with 5% sheep blood is red and opaque.

Expected Cultural Response: Cultural response on Columbia CNA Agar at 35 ± 2°C after 18 - 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Hemolysis
Pseudomonas aeruginosa ATCC® 27853	~10^3	Completely Inhibited	-
Staphylococcus aureus ATCC® 25923	10 - 300	Good	Beta hemolysis
Streptococcus pneumoniae ATCC® 6305	10 - 300	Good	Alpha hemolysis
Streptococcus pyogenes ATCC® 19615	10 - 300	Good	Beta hemolysis

The organisms listed are the minimum that should be used for quality control testing.

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Test Procedure

1. Inoculate specimens directly onto surface of the medium. Streak for isolation with inoculating loop and stab the agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to activity of both oxygen-stable and oxygen-labile streptolysins.
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime-hemolysis (α) is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human, and rabbit blood agar andalpha-hemolytic on sheep blood agar.
2. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci. For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%) in accordance with established laboratory procedures.
3. *Proteus* spp. occasionally grow on CNA Agar and may initially be confused with streptococci because of the small size of the colonies.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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