Columbia Blood Agar Base CE (NCM2023)

Intended Use
Columbia Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Description
A modification of the original Columbia Agar base formulation, Columbia Blood Agar Base provides a medium that is suitable for use with both defibrinated horse or sheep blood. The peptone mixture provides a source of nitrogen, essential vitamins and amino acids. The starch provides a carbon source and sodium chloride maintains osmotic balance. Originally described as a general purpose nutritious agar base by Ellner *et al.* Columbia Agar is more frequently used when enriched by the addition of sterile blood. The medium is suitable for supporting the growth and determining hemolytic reactions of a variety of microorganisms.

Typical Formulation

Columbia peptone mixture	25.1 g/L
Soluble starch	1.0 g/L
Sodium chloride	5.0 g/L
Agar	12.0 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
1. Refer to SDS

Preparation
1. Suspend 43.1 grams 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.
4. Cool to 45-50°C and add 5-7% sterile defibrinated horse or sheep blood.
5. Mix well before dispensing into sterile Petri dishes.

Test Procedure
1. Process each sample as appropriate and inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop, and stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the 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.

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

Prepared Appearance: Prepared medium without blood is light amber, and clear. With 5% sheep or horse blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Columbia Blood Agar Base with 5% defibrinated sheep or horse blood at 37± 1ºC after 18 - 48 hours incubation.

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Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Hemolysis
Escherichia coli ATCC® 25922	50-200	> 70%	Beta hemolysis
Staphylococcus aureus ATCC® 25923	50-200	> 70%	Beta hemolysis
Streptococcus pneumoniae ATCC® 6305	$>10^4$	4 Quad streak	Alpha hemolysis
Streptococcus pyogenes ATCC® 19615	50-200	> 70%	Beta hemolysis
Enterococcus faecalis ATCC® 29212	$>10^4$	4 Quad streak	N/A

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

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. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. 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 and alpha-hemolytic on sheep blood agar.
3. 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.

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

1. Ellner, P.D., Stoessel, C.J., Drakeford, E. and Vasi, F. (1966). A new culture medium for medical bacteriology. *Amer J. Clin Pathol.* **45**. 502-504.

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