COLUMBIA AGAR WITH 5% SHEEP BLOOD

INTENDED USE: for the isolation, cultivation and determining hemolytic reactions of fastidious pathogenic microorganisms.

For in vitro diagnostics use only.

SUMMARY AND EXPLANATION OF THE TEST: Columbia Agar supplemented with 5% sheep blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms.
Columbia Blood Agar Base was developed after the Columbia Agar formulation described by Elner et. al. from Columbia University.[1] Columbia (Blood Agar Base) BAB is specified in the Compendium of Methods for the Microbiological Examination of Food.[2]

PRINCIPLES OF THE PROCEDURE: The nitrogen, vitamin, and carbon, sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, and Yeast Enriched Peptone. Corn Starch increases growth of *Neisseria* spp., and enhances the hemolytic reactions of some streptococci. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.
In General, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β-hemolytic streptococci.[3] Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[4]

FORMULA/LITER:

Enzymatic Digest of Casein	5,0 g
Enzymatic Digest of Animal Tissue	8,0 g
Yeast Enriched Peptone	10,0 g
Agar	14,0 g
Sodium Chloride	5,0 g
Corn Starch	1,0 g

SUPPLEMENTS:

Sheep blood	50,0 ml

FINAL pH: 7,3 ± 0.2 at 25°C.

DIRECTIONS FOR PREPARATION FROM DEHYDRATED PRODUCT: Suspend 43 g of the medium in one liter of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes. Prepare 5% blood agar by aseptically adding the 50 ml of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

PROCEDURE: Process each specimen 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.[4] Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

EXPECTED 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:[5]

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.

QUALITY CONTROL SPECIFICATIONS:
Dehydrated Appearance: Powder is homogeneous, free flowing and beige.
Prepared Appearance: Prepared medium without blood is light to medium amber, and clear to moderately hazy. With 5% sheep blood the medium is red and opaque.
Expected Cultural Response: Cultural response on Columbia Blood Agar Base at 35°C after 18 - 24 hours incubation.

Microorganism	Response (Plain and with 5% Sheep Blood)	Reactions (With 5% Sheep Blood)
Escherichia coli ATCC® 25922	growth	---
Staphylococcus aureus ATCC® 25923	growth	beta hemolysis
Streptococcus pneumoniae ATCC® 6305	growth	alpha hemolysis
Streptococcus pyogenes ATCC®19615	growth	beta hemolysis

STORAGE: Store sealed box containing the dehydrated medium at 2 - 30°C. 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. Ready to use plates should be stored at 6 - 12°C. Bottle containing Columbia Agar Base 6 - 25°C.

PRECAUTIONS: for laboratory use only.

PACKAGING:
cat No. 1190 ready to use plates (1x10 pcs);
cat No. 3014 bottle with Columbia Agar Base (100; 200; 500 ml);
cat No. 107 dehydrated medium (100; 250; 500 g);

EXPIRATION:
ready to use plates – 45 days;
bottle (base) – 1 year;
dehydrated medium – 3 years.

REFERENCES:

1. Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi. 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. 45:502-504.
2. Vanderzant, C., and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of food, 3rd., p. 1113. American Public Health Association, Washington, D.C.
3. Casman, E. P. 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. 17:281-289.
4. Ruoff, K. L. 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th. American Society for Microbiology, Washington, D. C.
5. Isenberg, H. D. (ed.). 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.

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