COLUMBIA BLOOD AGAR BASE (7125)

Intended Use

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

Product Summary and Explanation

Columbia blood agar base media are typically supplemented with 5-10% sheep, rabbit, or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, Columbia Blood Agar Base is used as a general purpose media.

Columbia Blood Agar Base was developed after the Columbia Agar formulation described by Ellner et al. from Columbia University.[1] Columbia (Blood Agar Base) BAB is specified in the Compendium of Methods for the Microbiological Examination of Foods.[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 g
Enzymatic Digest of Animal Tissue ..... 8 g
Yeast Enriched Peptone ..... 10 g
Corn Starch ..... 1 g
Sodium Chloride ..... 5 g
Agar ..... 14 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to skin, eyes, and mucous membranes

Directions

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.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

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

Prepared Appearance: Prepared medium without blood is light to medium amber, and light 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 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Hemolysis
Escherichia coli ATCC® 25922	10 - 300	Good to excellent	Beta hemolysis
Staphylococcus aureus ATCC® 25923	10 - 300	Good to excellent	Beta hemolysis
Streptococcus pneumoniae ATCC® 6305	10 - 300	Good to excellent	Alpha hemolysis
Streptococcus pyogenes ATCC® 19615	10 - 300	Good to excellent	Beta hazy after 18 – 24 hours / Beta clear after 40 – 48 hours

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

Test Procedure

1. 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]
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:[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.

Storage

Store sealed bottle 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.

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.[4]
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[4] For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Packaging

Columbia Blood Agar Base	**Code No.**	**7125A**	**500 g**
		7125B	**2 kg**
		7125C	**10 kg**

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 ed., 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 ed. 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.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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