COLUMBIA CNA AGAR (7126)

Intended Use

Columbia CNA Agar is used with blood for the selective isolation of Gram-positive cocci.

Product Summary and Explanation

Ellner et al. described Columbia CNA Agar as a variation of Columbia Blood Agar Base that is selective for Gram-positive cocci.[1] Colistin and Nalidixic Acid are added to the formula, selecting for Gram-positive organisms and fungi by suppressing gram-negative bacteria. Columbia CNA Agar is used as a primary plating medium for urine cultures.[3]

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.

Nalidixic Acid and Colistin are the antimicrobics suppressing the growth of *Enterobacteriaceae* and *Pseudomonas* spp., and allowing yeast, staphylococci, streptococci, and enterococci to grow.[4] Certain Gram-negative organisms, such as *Gardnerella vaginalis* and certain *Bacteriodes* spp., can grow very well on Columbia CNA Agar with blood.[4] Colistin disrupts the cell membrane of Gram-negative organisms, particularly effective against *Pseudomonas* spp.[2] Nalidixic Acid blocks DNA replication in susceptible bacteria and acts against many Gram-negative bacteria.[2]

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
Colistin ..... 0.015 g
Nalidixic Acid..... 0.01 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 eyes, skin, and respiratory system.

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. Do not overheat medium.
4. Prepare 5% 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 moderately hazy and light to medium amber. With 5% sheep blood the medium is red and opaque.

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

Microorganism	Response	Reactions
Pseudomonas aeruginosa ATCC® 27853	inhibited	-
Staphylococcus aureus ATCC® 25923	growth	beta hemolysis
Streptococcus pneumoniae ATCC® 6305	growth	alpha hemolysis
Streptococcus pyogenes ATCC® 19615	growth	beta hemolysis

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

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.[5]
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:[3]

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.[5]
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[5] For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%) in accordance with established laboratory procedures.
4. *Proteus* spp. occasionally grow on CNA Agar and may initially be confused with streptococci because of the small size of the colonies.[2]

Packaging

Columbia CNA Agar	**Code No.**	**7126A**	**500 g**
		7126B	**2 kg**
		7126C	**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. **Estevez, E. G.** 1984. Bacteriological plate media: review of mechanisms of action. Lab. Med. **15**:258-262.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
5. **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.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.