Columbia Agar (NCM0013)

Revision: 2 Effective Date: 12/1/2021

Intended Use
Columbia Agar is used with or without blood for the isolation and cultivation of a wide variety of fastidious microorganisms. Conforms to Harmonized USP/EP/JP Requirements. Columbia Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description
A medium recommended by the Harmonized USP/EP/JP for isolation and identification of *Clostridia* from non-sterile products. Conforms to Harmonized USP/EP/JP performance specification. Originally described as a general purpose nutritious agar base by Ellner *et al.* at Columbia University that can be enriched by the addition of sterile blood. The peptone mixture and yeast extract provide a source of nitrogen, essential vitamins and amino acids. The starch provides a carbon source and sodium chloride maintains osmotic balance. The Harmonized European Pharmacopoeia states that where necessary, gentamicin sulfate at a concentration of 20mg/L can be added after sterilization to reduce the growth of non-target organisms. According to the Harmonized European Pharmacopoeia, Reinforced Medium for Clostridia is used as a selective enrichment broth, with subculture performed onto Columbia Agar.

Typical Formulation

Pancreatic Digest of Casein	10.0 g/L
Meat Peptic Digest	5.0 g/L
Heart Pancreatic Digest	3.0 g/L
Yeast Extract	5.0 g/L
Maize 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
Refer to SDS

Preparation
1. Dissolve 41 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. Cool 45-50°C,

Test Procedure
According to the Harmonized US/EP/JP

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

Prepared Appearance: Prepared medium without blood is trace to very slightly hazy and light amber. With 5% sheep blood the medium is opaque and red.

Expected Cultural Response and USP/EP/JP Growth Promotion Testing: Columbia Agar was prepared according to label directions and inoculated with the organisms listed below. Cultures were incubated under appropriate atmosphere at 30 – 35° C and examined for growth at 18 – 48 hours.

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Without 5% defibrinated sheep blood:

Microorganism	Approx. Inoculum (CFU)	Expected Results
Escherichia coli ATCC® 8739	10-100	70-200%
Pseudomonas aeruginosa ATCC® 9027	10-100	70-200%
Staph aureus ATCC® 6538	10-100	70-200%
Streptococcus pyogenes ATCC® 12344	10-100	70-200%

With 5% defibrinated sheep blood:

Escherichia coli ATCC® 8739	10-100	70-200%
Listeria monocytogenes ATCC® 7644	10-100	70-200%
Staphylococcus aureus ATCC® 6538	10-100	70-200%
Streptococcus pneumoniae ATCC® 6305	10-100	70-200%
Streptococcus pyogenes ATCC® 12344	10-100	70-200%

This culture was tested at Harmonized USP/EP/JP specified temperatures and incubation times

Clostridium sporogenes coli ATCC® 19404	10-100	70-200%

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

Results

Examine Columbia Agar for growth.

For Columbia Agar, supplemented with blood, examine the medium for growth and hemolytic reactions after 18 – 24, and after 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 CO2 (5 - 10%) in accordance with established laboratory procedures.

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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. European Pharmacopoeia 10th Edition (2020)
2. United States Pharmacopeia National Formulary 2018: USP 41 NF 36
3. Japanese Pharmacopeia 17th Edition (2017)
4. 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.
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.
6. Casman, E. P. 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. 17:281-289.
7. 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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