COLUMBIA AGAR TMH 116

Selective and nutritive for *Clostridia* species

Composition

Ingredients	Gms/Ltr.
Agar	15.00
Pancreatic digest of Casein	10.00
Peptic digest of meat	5.00
Yeast extract	5.00
Sodium chloride	5.00
Pancreatic digest of heart	3.00
Maize starch	1.00

*Dehydrated powder, hygroscopic by nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct sunlight.

Instructions for Use

Dissolve 44.0gms in 1000ml of distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool the medium to 45-50°C. Columbia Agar can be supplemented with 5-10% sheep, rabbit, or horse blood for use in isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Mix well and pour the medium into sterile petri plates.

Appearance: Light amber colour, slightly opalescent gel, *With blood* -Cherish red colour.
pH (at 25°C): 7.3 ± 0.2

Principle

COLUMBIA AGAR is a highly nutritive and selective medium for the cultivation of fastidious organisms, especially when used as a base for blood chocolate agar. It can also be used as a selective isolation medium by adding antimicrobial agents. It is a nutritiously rich general purpose medium developed by Ellner et al[5] from Columbia University. Columbia Agar conforms to harmonized USP/EU/JP requirements.[1,2,3,4] Nitrogen, vitamins, and amino acids are provided by a combination of Pancreatic digest of Casein, Peptic digest of Meat and Pancreatic digest of Heart. Maize starch is included to supply a carbon source. Yeast extract provides B-complex vitamins. Sodium chloride maintains the osmotic balance of the medium. Agar acts as a solidifying agent. 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.[6] In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of hemolytic streptococci. Inoculate Columbia Agar (TMH 116) medium with a small number (not more than 100 cfu) of the appropriate microorganism and incubate under anaerobic conditions at 30 - 35°C for 18 - 48 hours. For isolation purpose, streak with inoculating loop on Columbia Agar (TMH 116) and incubate under anaerobic conditions at 30-35°C for 48 hours.
For Columbia Agar, supplemented with blood, examine the hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as[7]:

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.

Interpretation

Cultural characteristics observed after incubation at 30-35°C for 48 hours.

Microorganisms	ATCC	Inoculum (CFU)	Recovery Rate	Appearance	Hemolysis
Escherichia coli	25922	10-100	≥ 50%	Opaque yellow colour	Beta
Streptococcus pneumoniae	6305	10-100	≥ 70%	Light brown colour	Alpha
Staphylococcus aureus	25935	10-100	≥ 70%	Light yellow colour	Beta

References

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5. 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.
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