PRODUCT SPECIFICATION SHEET

Columbia Blood Agar Base (DM063)

Intended Use

Columbia Blood Agar Base is an efficient base medium for preparation of blood agar, chocolate agar and various selective and identification media.

Product Summary and Explanation

Ellner et al[1] in 1966, reported the development of a blood agar formulation, which has been designated as Columbia Agar. Columbia (Blood Agar Base) BAB is specified in the Compendium of Methods for the Microbiological Examination of Foods.[2] Columbia Agar Base is utilized as the base for media containing blood and for selective media formulations in which various combinations of antimicrobial agents are used as additives. 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. Sheep blood has been added for the observation of hemolytic reactions as seen by the double zone β-hemolysis of *Clostridium perfringens*. This medium is prepared, stored and dispensed under oxygen-free conditions to prevent the formation of oxidized products prior to use.

Principles of the Procedure

The nitrogen, vitamin, and carbon, sources are provided by the Special Peptone. Corn Starch increases growth of Neisseria spp., and enhances the hemolytic reactions of some Streptococci. Corn starch serves as an energy source and also neutralizes toxic metabolites. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. The special peptone supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both the X & V factors and the medium would support the growth of *H. influenza*.[6,7] The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of Haemophilus species from clinical specimens, especially from upper respiratory tract[8]. Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

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]

Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence Haemophilus influenzae which needs both the X and V factors, will not grow on this medium. As this medium have a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction which may be mistaken for the alpha haemolysis. Carry out confirmatory tests of all the colonies. Columbia Agar Base with added sterile serum provides an efficient medium for Corynebacterium diphtheriae virulence test medium. After following the established technique for C. diphtheriae, lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO2.

Precaution: Brucella cultures are highly infective and must be handled carefully; incubate in 5-10% CO2. Campylobacter species are best grown at 42°C in a microaerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2.[9]

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Formula / Liter

Ingredients	Gms / Liter
Peptone, special	23.00
Starch	1.00
Sodium chloride	5.00
Agar	15.00
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 only.
2. IRRITANT. Irritating to skin, eyes, and mucous membranes

Directions

1. Suspend 44 grams of in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.
2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.
3. For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.
4. For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.
5. The medium can be made selective by adding different antimicrobials to sterile base.
6. For Brucella species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (MS043) to 500 ml sterile molten base.
7. For Campylobacter species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (MS004) or Campylobacter Supplement- II, (Butzler) (MS005) or Campylobacter Supplement- III (Skirrow) (MS007) or Campylobacter Selective Supplement (MS155) or Campylobacter Supplement- VI (Butzler) (MS138) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement (MS008) and 5-7% v/v horse or sheep blood.
8. For Gardnerella species: Add rehydrated contents of 1 vial of G. Vaginalis Selective Supplement (MS018) to 500 ml sterile molten base.
9. For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (MS026) or Strepto Supplement (MS027) or Streptococcus Selective Supplement (MS027) to 500 ml sterile molten base.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow, homogeneous, free flowing powder
Prepared Medium	Basal medium: Light amber coloured clear to slightly opalescent gel.
	After addition of 5% w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates
Reaction of 4.4% Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 24-48 hours..

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Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Haemolysis
1.	*Neisseria meningitidis ATCC 13090*	50 -100	Luxuriant	>=70%	None
2.	*Staphylococcus aureus ATCC 25923*	50 -100	Luxuriant	>=70%	Beta / gamma
3.	*Staphylococcus aureus ATCC 6538*	50-100	Luxuriant	>=70%	Beta / gamma
4.	*Staphylococcus aureus NCIMB 9518*	50-100	Luxuriant	>=70%	Beta / gamma
5.	*Staphylococcus epidermidis ATCC 12228*	50-100	Luxuriant	>=70%	Gamma
6.	*Streptococcus pneumoniae ATCC 6303*	50 -100	Luxuriant	>=70%	alpha
7.	*Streptococcus pyogenes ATCC 19615*	50 -100	Luxuriant	>=70%	Beta
8.	*Clostridium sporogenes ATCC 19404*	50-100	Luxuriant	>=70%	--
9.	*Clostridium sporogenes ATCC 11437*	50-100	Luxuriant	>=70%	--
10.	*Clostridium perfringens ATCC 13124*	50-100	Luxuriant	>=70%	--
11.	*Clostridium perfringens ATCC 12934*	50 -100	Luxuriant	>=70%	--

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 CO2 (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: [10]

a. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
b. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
c. Alpha-prime-hemolysis (α') is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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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.[10]
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

Product Name : Columbia Blood Agar Base
Product Code : DM063
Available Pack sizes : 100gm / 500gm

References

1. Ellner, Stoessel, Drakeford and Vasi. 1966. Am. J. Clin. Pathol. 45:502.
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.
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6. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
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8. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
9. Bailey R. K., Voss J. L. and Smith R. F., 1979, J. Clin. Microbiol., 9 ; 65-71
10. 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.

Further Information

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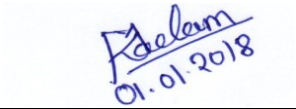	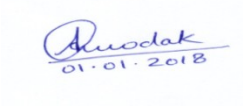	
Microbiologist	Head Quality Control	Head Quality Assurance