Technical Information

Columbia Blood Agar Base

Product Code: DM 1144

Application: - Columbia Blood Agar Base is used as an efficient base for preparation of blood agar, chocolate agar and for preparation of various selective and identification media.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Ellner et al [1] devised Columbia Blood Agar Base was devised. This medium contains special peptone which 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 support the growth of H. influenzae [2, 3]. 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 [4]. Columbia Agar Base is used as base media containing blood and selective media formulations with different combinations of antimicrobial agents for isolation of different patriogons as reported in the literature.

Corn starch serves as an energy source and also neutralizes toxic metabolites. 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. Columbia Agar Base with added sterile serum provides an efficient medium for Corynebacterium diphtheriae virulence test medium, and 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% CO_2.

Precaution: Brucella cultures are highly infective and must be handled carefully; incubate in 5-10% CO_2. 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 [5]

Methodology

Suspend 44 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.

For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.

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. The medium can be made selective by adding different antimicrobials to sterile base.

For Brucella species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (MS2005) to 500 ml sterile molten base.

For Campylobacter species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (MS2006) or Campylobacter Supplement- II, (Butzler) (MS2007) or Campylobacter Supplement- III (Skirrow) (MS2008) or Campylobacter Selective Supplement (MS2090) or Campylobacter Supplement- VI (Butzler) (MS2106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement (MS2009) and 5-7% v/v horse or sheep blood.

For Gardnerella species: Add rehydrated contents of 1 vial of G.Vaginalis Selective Supplement (MS2056) to 500 ml sterile molten base.

For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (MS2030) or Strepto Supplement (MS2031) or Streptococcus Selective Supplement (MS2119) to 500 ml sterile molten base.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of 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
Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH range 7.10-7.50

Growth Promotion Test
In accordance with the harmonazied method of USP/EP/BP/JP.

Cultural Response/ characteristices
DM1144: Cultural characteristics observed with added 5% w/v sterile defibrinatedblood, after an incubation at 35-37°C for 24-48 hours..

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus aureus ATCC 6538	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus aureus ATCC 9518	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus epidermidis ATCC 12228	50-100	inhibited	>=70%	gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta
Clostridium sporogenes ATCC 19404	50-100	luxuriant	>=50%	
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%	
Clostridium perfringens ATCC 13124	50-100	luxuriant	>=50%	
Clostridium perfringens ATCC 12934	50-100	luxuriant	>=50%	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
2. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
3. Fildes P., 1921, Br. J. Exp. Pathol., 2:16.
4. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
5. Bailey R. K., Voss J. L. and Smith R. F., 1979, J. Clin. Microbiol., 9 ; 65-71

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