Columbia Blood Agar Base w/ Hemin

M1133

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

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Hemin	0.010
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.01 grams in 1000 ml distilled water. Heat to boiling 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 (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (FD006) or Campylobacter Supplement- II, (Butzler) (FD007) or Campylobacter Supplement- III (Skirrow) (FD008) or Campylobacter Selective Supplement (FD090) or Campylobacter Supplement- VI (Butzler) (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement (FD009).

For *Gardnerella* species: Add rehydrated contents of 1 vial of G. Vaginalis Selective Supplement (FD056) to 500 ml sterile molten base.

For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (FD030) or Strepto Supplement (FD031) or Streptococcus Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

Columbia Agar Base is used as the base for media containing blood and for selective media formulations, which incorporates various combinations of antimicrobial agents as additives. Sheep blood allows detection of hemolytic reactions and supplies the X-factor (hemin) necessary for the growth of many bacterial species but lacks V-factor (Nicotinamide Adenine Dinucleotide), since it contains NADase, which destroys the NAD. Therefore, *Haemophilus influenzae* , which requires both the X and V-factors, will not grow on this medium. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would 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 Blood Agar Base w/ 1 % Agar is used as a base for preparing media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Columbia Agar Base supplemented with sheep, rabbit or horse blood derives its superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef extract. Cornstarch 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. As these media have a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction, which may be mistaken for alpha haemolysis. Confirmatory tests of all the presumptive colonies are needed.

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% CO2 (5).

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, X and V factors will not grow on this medium. Hemin stimulates growth of various fastidious organisms. As this medium has 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.

Quality Control

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.On standing the molten medium shows haziness.
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
7.10-7.50

Cultural Response
M1133: Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Cultural Response				
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	>=70%	gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta

Storage and Shelf Life

Store below 30°C and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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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