

Columbia Blood Agar Base**M144**

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

Directions

Suspend 44 grams of 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) and 5-7% v/v horse or sheep blood.

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 Blood Agar Base was devised by Ellner et al (1). 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 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 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.

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. 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 microaerophilic atmosphere. Plates with *Gardenerella* supplements plates should be incubated at 35°C for 48 hours containing 7% CO₂ (5)

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. 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

Growth Promotion Test

In accordance with the harmonized method of USP/EP/BP/JP.

Cultural Response

M144: Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 24-48 hours. (Clostridium species incubated under anaerobic conditions at 35-37°C for 24-48 hours) .

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis		
<i>Neisseria meningitidis</i> ATCC 13090	50-100	luxuriant	≥70%	none		
<i>Staphylococcus aureus</i> ATCC 25923	50-100	luxuriant	≥70%	beta / gamma		
<i>Staphylococcus aureus</i> ATCC 6538	50-100	luxuriant	≥70%	beta / gamma		
<i>Staphylococcus aureus</i> NCIMB 9518	50-100	luxuriant	≥70%	beta / gamma		
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	luxuriant	≥70%	gamma		
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	luxuriant	≥70%	alpha		
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	luxuriant	≥70%	beta		
<i>Clostridium sporogenes</i> ATCC 19404	50-100	luxuriant	≥50%			
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	≥50%			
<i>Clostridium perfringens</i> ATCC 13124	50-100	luxuriant	≥50%			
<i>Clostridium perfringens</i> ATCC 12934	50-100	luxuriant	≥50%			
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	≥70%	beta		
<i>Escherichia coli</i> ATCC 8739	50-100	luxuriant	≥70%	beta		
<i>Escherichia coli</i> NCTC 9002	50-100	luxuriant	≥70%	beta		

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

Storage and Shelf Life

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