Columbia Blood Agar Base, HiVeg®

MV144

Intended Use:

It is used as an efficient base for preparation of blood agar, chocolate agar and for various selective and identification media.

Composition**

Ingredients	g / L
HiVeg® special peptone	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 44.0 grams of in 1000 ml purified/ 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 NPBCVN Selective Supplement (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement(FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) or VTCA Selective Supplement (FD090) or Butzler VI Selective Supplement (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Minerals Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.

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

For Cocci: Add rehydrated contents of 1 vial of NC Selective Supplement (FD030) or NNP Selective Supplement (FD031) or CO Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

Columbia Blood Agar Base, HiVeg® Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. 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.

The medium contains HiVeg® special peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. 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. 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% CO_2. *Campylobacter* species are best grown at 42°C in a micro aerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2 (2).

Type of specimen

Clinical samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. 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.
3. Carry out confirmatory tests of all the colonies.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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
pH
7.10-7.50
Growth Promotion Test
In accordance with the harmonazied method of USP/EP/BP/JP.
Cultural Response
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 subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	beta / gamma

Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	gamma
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	luxuriant	>=70%	beta / gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	>=50 %	
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50 %	
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 12934	50-100	luxuriant	>=50 %	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

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. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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