

Columbia Blood HiCynth™ Agar Base

MCD144

Intended Use:

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**

	Gms / Litre
HiCynth™ Peptone No.3*	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

*Chemically defined peptone

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 w/1% Agar is a general-purpose nutritious agar base formulated by Ellner et al (1) in 1966; it is further enriched by the addition of sterile blood. Traditionally blood agar bases have incorporated either casein hydrolysate to give rapid production of large colonies or meat infusion to give defined hemolytic reactions. The media combines both to give an improved all round performance.

Columbia Blood HiCynth™ Agar Base w/1% Agar is the modification of the same, but prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. This medium with the added HiCynth™ Peptone No.3 supports rapid and luxuriant growth of fastidious and nonfastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. 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 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.

Columbia Blood HiCynth™ Agar Base w/1% Agar 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. HiCynth™ Agar Base w/1% Agar 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 should be incubated at 35°C for 48 hours containing 7% CO₂ (2).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3). 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 greenish haemolytic reaction which may be mistaken for the alpha haemolysis.
3. Carry out confirmatory tests of all the colonies.
4. Due to variable nutritional requirements some species may show variable growth pattern.

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

Cultural Response

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
<i>Neisseria meningitidis</i> ATCC 13090	50-100	luxuriant	≥70%	none
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	luxuriant	≥70%	beta / gamma
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	luxuriant	≥70%	gamma

<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	luxuriant	>=70%	beta / 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 (00008*)	50-100	luxuriant	>=50 %	
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	>=50 %	
<i>Clostridium perfringens</i> ATCC 13124 (00007*)	50-100	luxuriant	>=50 %	
<i>Clostridium perfringens</i> 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 (4,5).

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. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. 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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Disclaimer :

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