Technical Data

Columbia Blood HiCynth™ Agar Base w/ 1% Agar MCD144A

Columbia Blood HiCynth™ Agar Base w/1% Agar is used with blood for isolation and cultivation of fastidious bacteria.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.3*	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	10.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptone

Directions

Suspend 39 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. Mix well an pour into sterile Petri plates.

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 Blood Agar Base is a general-purpose nutritious agar base formulated by Ellner et al (1) in 1966 and enriched by the addition of sterile blood. Traditionally blood agar bases have incorporated either casein hydrolysate or meat infusion, to give rapid production of large colonies to give defined hemolytic reactions. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Columbia HiCynth™ Agar Base is prepared by replacing animal or vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. It 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. Fildes found that Nutrient Agar supplemented with 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 medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Blood HiCynth™ 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.

HiCynth™ Peptone No.3 provides nitrogen and carbon compounds, long chain amino acids vitamins and other growth factors. 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 HiCynth™ 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 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 1.0% Agar gel.

Colour and Clarity of prepared medium

Basal medium :Light amber coloured clear to slightly opalescent gel. After addition of 5%v/v sterile defirinated blood : Reddish brown coloured opaque gel forms in Petri plates

Reaction

Reaction of 3.9% 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 40-48 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 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 in tightly closed container 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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