Technical Information

Columbia Blood Agar Base w/ Hemin

Product Code: DM 2133

Application: - 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

Principle & Interpretation

Columbia Agar Base is used as the base for media contai ning 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 bot h 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 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 so urce 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 on all the presumptive colonies are needed to establish diagnosis.

Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium in which *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% CO_2.

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 microaerophillic atmosphere. Plates with *Gardenerella* supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2 [5].

Methodology

Suspend 44.01 grams of powder media in 1000 ml distilled water. Shake well & heat 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 const ant 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 (MS2005) to 500 ml sterile molten base. For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (MS2006) or Campylobacter Supplement- II, (Butzler) (MS2007) or Campylobacter Supplement- III (Skirrow) (MS2008) or Campylobacter Selective Supplement (MS2090) or Campylobacter Supplement- VI (Butzler) (MS2106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Cam pylobacter Growth Supplement (MS2009).
For *Gardnerella* species: Add rehydrated contents of 1 vial of G. Vaginalis Selective Supplement (MS2056) to 500 ml sterile molten base.
For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (MS2030) or Strepto Supplement (MS2031) or Streptococcus Selective Supplement (MS2119) to 500 ml sterile molten base.

Quality Control

Physical 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 range 7.10-7.50

Cultural Response/ characteristices

DM 2133: 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
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

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specificatons for identity and performens parameters.