Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Columbia C.N.A. Agar Base

Product Code: DM 1560

Application: - Columbia Broth Base is used as a general-purpose medium and also for the cultivation of fastidious organisms.

Composition**

Ingredients	Gms / Litre
Biopeptone	20.000
Tryptic digest of beef heart	3.000
Corn starch	1.000
Sodium chloride	5.000
Colistin sulphate	0.010
Nalidixic acid	0.015
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Columbia Blood Agar Base is utilized as a base for preparation of media containing blood and in selective media preparations where various combinations of antimicrobial agents are used as additives. Ellner et al formulated the medium [(1)] and found that the combination of peptones used gave more rapid and abundant growth of Streptococci, Staphylococci, *Neisseria* and *Haemophilus* with better-defined haemolytic reactions. Columbia C.N.A. Agar Base is prepared with the same formula as Columbia Agar Base with the addition of 10 mg/litre of colistin and 15 mg/ litre of nalidixic acid to inhibit the growth of gram-negative bacteria and to support the growth of Staphylococci, haemolytic Streptococci and Enterococci when supplemented with 5% blood.

Biopeptone and tryptic digest of beef heart supports luxuriant growth of microorganisms and visualization of good haemolytic reactions. Sheep blood allows detection of haemolytic reactions and supplies both X necessary for the growth of many bacterial species. Horse blood supplies Both X- and V-factor, therefore is mostly preferred in most laboratories. Yeast extract and cornstarch serve as energy source and neutralizer respectively.

It should be noted that this medium has relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha haemolysis. The addition of the antimicrobial agents, colistin (or polymyxin B) and nalidixic acid, renders the medium selective for gram-positive microorganisms [(2).] Colistin and nalidixic acid disrupt the cell membrane of gram-negative organisms, whereas nalidixic acid blocks DNA replication in susceptible gram-negative bacteria [(3).]

Columbia C.N.A. Agar Base with addition of blood used for selective isolation of *Gardnerella vaginalis.* This medium supports growth of *Brucella abortus, Yersinina pestis, Clostridium perfringens* and all commonly occurring *Enterobacteriaceae* without addition of blood.

Methodology

Suspend 44.02 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 50°C and aseptically add 5% v/v sterile, defibrinated blood. Mix well and pour into sterile Petri plates.

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: Yellow coloured clear to slightly opalescent gel. After addition of 5% v/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

DM 1560: Cultural characteristics observed with added 5% v/v sterile, defibrinated blood after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Neisseria meningitidis ATCC 13090	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=50%	beta/gamma
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	>=50%	gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=50%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=50%	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 et al, 1966, Am. J. Clin. Path., 45:502.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3. Estevez, 1984, Lab. Med., 15:258

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