Columbia C.N.A. Agar Base

M560

Intended Use:

Recommended for selective isolation of pathogenic Gram-positive cocci from clinical and nonclinical specimens.

Composition**

Ingredients	Gms / Litre
Biopeptone	20.000
Tryptose B	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

Directions

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

Principle And Interpretation

Columbia Agar Base is a nutritionally rich formula containing 5% defibrinated blood, which provides more nutrients and capability of displaying haemolytic reactions. 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 tryptose B supports luxuriant growth of microorganisms and visualization of good haemolytic reactions. Sheep blood allows detection of haemolytic reactions and supplies X-factor necessary for the growth of many bacterial species. Horse blood supplies X-factor 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 gives selective isolation of gram-positive cocci, Staphylococci and Streptococci, particularly when gram-negative bacilli are present and tend to overgrow on conventional blood agar plates. Also 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.

Type of specimen

Clinical samples - Blood, skin scrapings, etc; Dairy samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some beta-hemolytic streptococci strains will develop hemolytic zones on Columbia CNA Agar. It is recommended to subculture all such streptococci strains onto Blood Agar (M073) for further identification.
2. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the d oh od hnc 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: 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

7.10-7.50

Cultural Response

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	>=10^4	inhibited	0%	
Neisseria meningitidis ATCC 13090	>=10^4	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

Reference

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

Revision : 02 / 2015