Anaerobic CNA Agar Base

M1034

Intended Use:

Recommended for selective isolation of anaerobic Streptococci.

Composition**

Ingredients	Gms / Litre
Tryptone	12.000
Peptone	5.000
Yeast extract	3.000
HM peptone B #	3.000
Corn starch	1.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Dithioerythreitol (DTE)	0.100
L-Cystine hydrochloride	0.500
Vitamin K1	0.010
Hemin	0.010
Colistin	0.010
Nalidixic acid	0.010
Agar	13.500

**Formula adjusted, standardized to suit performance parameters
- Equivalent to Beef extract

Directions

Suspend 44.14 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in 100 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5 ml sterile defibrinated sheep blood to every 100 ml medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The genus *Streptococcus* is comprised of a wide variety of both pathogenic and commensal gram-positive bacteria, which are found to inhabit a wide range of hosts, including humans, horses, pigs and cows. They are facultatively anaerobic. Within the host, Streptococci are often found to colonize the mucosal surfaces of the mouth, nares and pharynx. However, in certain circumstances, they may also inhabit the skin, heart or muscle tissue. Streptococci are generally considered as fastidious organisms as they have exacting nutritional requirements. Columbia Agar formulated by Ellner et al. was designed to obtain luxuriant growth of various fastidious organisms (1). The media was rendered selective by the addition of selective agents, colistin (C) and nalidixic acid (NA). This supplemented Columbia Agar (with C & NA) exhibited luxuriant growth of fastidious organisms like Streptococci, Enterococci, and Staphylococci etc. on supplementation with sterile defibrinated sheep blood. Anaerobic CNA Agar Base is a modification of Columbia CNA Agar base with additional enrichment supplements i.e. vitamin K1 and hemin (2).

Columbia CNA Agar Base is used for the selective isolation of anaerobic gram-positive cocci including Streptococci. Tryptone, peptone, yeast extract and meat extract B serve as source of carbon, nitrogen, and essential nutrients. Corn starch neutralizes the toxic metabolites formed. Dextrose serves as the carbon source while sodium chloride maintains the osmotic equilibrium. Dithiothreitol and L- cystine help to create anaerobic conditions. Vitamin K1 and hemin stimulate growth of anaerobic bacteria. Colistin and Nalidixic acid in the medium inhibit accompanying gram-negative enteric bacteria (1) by disrupting the cell membrane and blocking DNA replication respectively (3).
Anaerobic CNA Agar plates should ideally be reduced prior to inoculation by keeping under anaerobic conditions for 18-24 hours. Samples can be directly streaked on the plates. Incubation of inoculated plates should be carried out at 35-37°C under anaerobic conditions for 48 hours. Negative cultures should be incubated for 7 day before reporting.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.35% 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 sheep blood: Cherry red coloured, opaque gel forms in Petri plates

Cultural Response

Cultural characteristics observed under anaerobic condition with added 5%v/v sterile defibrinated sheep blood,after an incubation at 35-37°C for 2-7 days.

Organism	Growth
Escherichia coli ATCC 25922	none-poor
Peptostreptococcus anaerobius ATCC 27337	good

Reference

1. Ellner, Stoessel, Drakeford and Vasi, 1966, Am. J. Clin. Pathol., 40. 502
2. Ellner, Granato and May, 1973, Appl.Microbiol. 26:904
3. Esteve Z. 1984, Lab Med., 15:258

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