

Technical Information

Anaerobic CNA Agar Base

Product Code: DM 2034

Application: - Anaerobic CNA Agar is used for the selective isolation of anaerobic Streptococci.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	12.000
Peptic digest of animal tissue	5.000
Yeast extract	3.000
Beef extract	3.000
Corn starch	1.000
Dextrose	1.000
Sodium chloride	5.000
Dithiothreitol (DTE)	0.100
L-Cystine hydrochloride	0.500
Vitamin K1	0.010
Hemin	0.010
Colistin	0.010
Nalidixic acid	0.010
Agar	
**Formula adjusted, standardized to suit performance parameters	13.500

Principle & 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 in mucosal surfaces of the mouth, nares and pharynx. However, in certain circumstances, they may also colonize 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 made selective by the addition of agents, like colistin (C) and nalidixic acid (NA). This supplemented Columbia Agar (when supplemented) with C & NA along with sterile defibrinated sheep blood, exhibited luxuriant growth of fastidious organisms like Streptococci, Enterococci, and Staphylococci etc. 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. Casein enzymic hydrolysate, peptic digest of animal tissue, yeast extract and beef extract 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 the growth of gram-negative enteric bacteria by disrupting the cell membrane and blocking DNA replication respectively^(1,3). Prior to inoculation anaerobic CNA Agar plates should ideally be exposed to 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.

Methodology

Suspend 44.14 grams of powder media in 1000 ml distilled water. Shake well and heat to boiling to dissolve the medium completely. Dispense in 100 ml amounts and sterilize by autoclaving at 15 lbs pressure (12 1°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.

Quality Control

Physical 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/Characteristics

DM 2034: 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

ATCC25922

none-poor

Peptostreptococcus anaerobius ATCC

27337

good

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^o in sealable plastic bags for 2-5 days.

Further Reading

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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