# Anaerobic CNA Agar Base

**M1034**

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 | 13.500 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 44.14 grams in 1000 ml 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.

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 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**
M1034: 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 |

## Storage and Shelf Life

Store dehydrated powder and prepared medium at 2-8°C in tightly closed container. Use before expiry date on label.

## 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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