

## CAA PRESUMPTIVE BROTH

**CAT Nº: 1209**

For the presumptive detection of intestinal enterococci in foods according to Mossel

### FORMULA IN g/l

Tryptone	20.00	Esculin	1.00
Yeast Extract	5.00	Ferric Ammonium Citrate	0.50
Sodium Chloride	5.00	Sodium Azide	0.15
Sodium Citrate	1.00	Kanamycin Sulfate	0.02

**Final pH 7.0 ± 0.2 at 25°C**

### PREPARATION

Suspend 33 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The medium should be stored at 2-8°C. The color is tournasol.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Caution: This medium is toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

### USES

CAA PRESUMPTIVE BROTH (Kanamycin, Aesculin, Azide) is a selective medium for the isolation of intestinal enterococci in foods. CAA Presumptive Broth is used in conjunction with CAA Confirmatory Agar (Cat. 1027).

Kanamycin, Sodium azide and Sodium citrate have a great inhibitory effect on the accompanying bacterial flora, they inhibit the growth of Gram positive and Gram negative bacteria, and the medium is highly selective for esculin-hydrolyzing enterococci. Esculin and Ferric Ammonium citrate are esculin indicators which detect the esculin-hydrolysing bacteria. They hydrolyze the glucoside esculin to give glucose and esculin. Tubes have a black color due to the reaction of the resulting esculin with the iron ions. Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

The presence of intestinal enterococci is an indicator for faecal en contamination, especially when the contamination occurred long ago and the less resistant coliform bacteria, including *Escherichia coli*, are already dead when the analysis is carried out.

Inoculate 1 ml of sample in tubes with 9 ml of single-strength medium. Always use 5 tubes for the MPN count technique. Incubate at 35 ± 2°C for 24 - 48 hours. Presumptive positive tubes have a brown-black color. This medium is recommended by CeNAN for food and drinks analysis.

Confirmatory tests, e.g. catalase test, glucose utilisation and growth at 45 °C ± 1 °C, may be carried out.

### MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24-48 hours.

Microorganisms	Growth	Esculin hydrolysis
<i>Enterococcus faecalis</i> ATCC 11700	Good	+
<i>Enterococcus faecium</i> ATCC 8043	Good	+
<i>Staphylococcus aureus</i> ATCC 6538	Moderate	
<i>Escherichia coli</i> ATCC 11775	Inhibited	-
<i>Lactococcus lactis</i> ATCC 19435	Moderate-Inhibited	

## BIBLIOGRAPHY

M.R. Pascual Anderson Técnicas para Examen Microbiológico de Alimentos y Bebidas (Centro Nacional de Alimentación y Nutrición CeNAN) Madrid, 1982Corps pag. 76 Aleman  
Brandl, E. Aspergerger H., Pflieger, F. U-IBEN CH: Zum Vorkomment von D-streptokokken in Käse. 1985..

## STORAGE

Once opened keep powdered medium closed to avoid hydration.

