

PRODUCT CODE: 417153

## CCA Coliforms, Chromogenic Agar Base (ISO 9308-1) (Dehydrated media) for microbiology

### Preparation

Suspend 29,45 g of powder in 1 L of distilled water and bring to the boil until fully dissolved. Do not autoclave nor overheat. Mix well and distribute into Petri dishes avoiding bubble formation.

### Uses

The combined action of peptone, yeast extract, pyruvate and sorbitol allow rapid colony growth in this phosphate buffered medium, which also permits simple recovery of sublethal thermally injured coliforms. Sodium chloride provides the correct osmotic environment necessary for growth. The selectivity is attained, partially, by the Tergitol® 7, which inhibits the growth of Gram positive bacteria and some Gram negative without effecting the coliform bacteria. Selectivity is enhanced by the cefsulodin and Vancomycin that which acts against pseudomonas and Gram-negative oxidase positive bacteria enterococci and other Gram positive bacteria. The culture medium was formulated without antibiotics for water samples with low bacterial background flora. The colonial differentiation is due to the chromogenic mixture, composed of two enzyme substrates: 6-chloro-3-indoxyl- $\beta$ -D-galacto-pyranoside (Salmon®-GAL) and 5-bromo-4-chloro-3-indoxyl- $\beta$ -D-glucuronide (X-Glucuronide). The first one is cleaved by the characteristic enzyme found in coliforms,  $\beta$ -D-galactosidase and gives a salmon-red colour to the coliform colonies. The second chromogenic substance is cleaved by the  $\beta$ -D-glucuronidase enzyme characteristic of *E. coli* and turns the colonies of these bacteria a blue colour. *E. coli* has the two enzymes and cleaves both chromogenic substances giving dark blue to violet colonies. Total coliforms are the sum of *E. coli* colonies plus salmon-red colonies. The IPTG enhances the metabolism of chromogenics. Other Gram-negative bacteria produce colourless colonies except some that possess glucuronidase activity (but not galactosidase) and they produce light blue to turquoise colonies. To confirm the *E. coli* colonies in this medium a small amount of tryptophan is included verifying indol production: coat the blue-violet colonies with a drop of Kovacs Reagent. If the reagent turns a cherry-red colour in a few seconds this confirms the production of indol and hence the presence of *E. coli*. When the Chromogenic Agar for Coliform is used with the membrane filter method, the colour and growth of the colonies can be modified by the characteristics of the membrane filter. It is advisable to perform validation of the membrane filter type used.

### Technique

The water sample is filtered through a membrane filter of 0.45  $\mu$ m pore diameter validated according to the ISO Standard 7704:1985. The membrane is then placed on the surface of the CCA medium avoiding entrapment of air bubbles between the membrane and agar surface.

The petri dish with the membrane is incubated for 18-24 hours at  $36 \pm 2^\circ\text{C}$ . If in 18 h there is growth of red or colourless colonies, extend the incubation until 24 h to include late reactions of  $\beta$ -galactosidase or  $\beta$ -glucuronidase. Count  $\beta$ -galactosidase positive colonies and  $\beta$ -glucuronidase negative colonies (all colonies coloured from salmon-rose to red) as Coliform bacteria not- *E. coli*. Count  $\beta$ -galactosidase positive colonies and  $\beta$ -glucuronidase positive colonies (all colonies coloured from deep blue to violet) as *E. coli*. Total Coliform count is obtained by the addition of the salmon-rose to red colonies plus the deep blue to violet colonies. Calculate the concentration of Coliform bacteria and *E. coli* in 100 mL from the initial volume of water filtered and the number of characteristic colonies counted on the membrane. The results are expressed as Colony Forming Units per millilitre (CFU/mL).

## Composition

See in Data Sheet (TDS).

## Microbiological Test

Incubation temperature: 36°C ±2.0

Incubation time: 18-24 h

Inoculum: Practical range 100±20 CFU. Min. 50 CFU (Productivity). MF Method. according to ISO 11133:2014

Microorganism	Growth	Colony Colour
<i>Escherichia coli</i> ATCC 8739	Productivity > 0.70	Blue-violet colonies. Indol (+)
<i>Escherichia coli</i> ATCC 25922	Productivity > 0.70	Blue-violet colonies. Indol (+)
<i>Citrobacter freundii</i> ATCC 43864	Productivity > 0.70	Salmon to red colonies. Indol (-)
<i>Pseudomonas aeruginosa</i> ATCC 10145	Good	Colorless colonies.
<i>Enterococcus faecalis</i> ATCC 19433	Inhibited	

## Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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