Product :

Microinstant ® CHROMOGENIC COLIFORMS AGAR BASE

Also known as

CCA; ACC

Specification

Selective and differential medium for the detection and enumeration of coliforms and *E. coli* in water samples by MF technique.

Formula * in g/L

Peptone	3.00	Sorbitol	1.00
Sodium chloride	5.00	6-Chloro-3-indoxyl-ß-D-galactopyranoside	0.20
Di-sodium hydrogen phosphate	2.70	5-Bromo-4-chloro-3-indoxyl-ß-D-glucuronic acid	0.20
Sodium dihydrogen phosphate dihydrate	2.20	Agar	13.00
Tryptophan	1.00		
Sodium pyruvate	1.00		
Tergitol®7	0.15	Final pH 6,8 ±0,2 at 25 ºC	

* Adjusted and /or supplemented as required to meet performance criteria

Directions

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. Cool until to 45-50ºC and add the contents of 2 vial of CV Selective Supplement for Coliforms (Art. No. 06 -140LYO1). Mix well and distribute into Petri dishes avoiding bubble formation. The finished plates remain effectives for at least one month if stored in the dark between 2-8ºC.

Description

The combined action of peptone, 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 colonial differentiation is due to the chromogenic mixture, composed of two enzyme substrates: 6-chloro-3-indoxyl-ß-D-galacto-pyranoside (Salmon®-GAL) and 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronide (X-Glucuronide).

The first one is cleaved by the characteristic enzyme found in coliforms, ß-D-galactosidase and gives a salmon-red colour to the coliform colonies. The second chromogenic substance is cleaved by the ß-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.

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 tryptophane 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.

The Spanish Health Ministry (Ministerio de Sanidad y Consumo) has officially adopted this medium as an alternative methodology for the microbiological analysis of water for human consumption, giving a new definition for *Escherichia coli* ("Enterobacteriaceae that express the ß-D- galactosidase and the ß-D-glucuronidase enzymes simultaneously") and coliform bacteria: "Enterobacteriaceae that express the ß-D-galactosidase enzyme".

Limitation of the procedure:

The production of β-galactosidase, although common to all the coliforms, varies from one strain to another being influenced by the temperature and incubation time. At temperatures above 37 ° C its production decreases, causing a loss of reddish color intensity, while the bluish tones in the strains of Escherichia coli are accentuated.

If the membrane filtration method is used, it must be taken into account that the nature and characteristics of the filter membrane used also influences the size and color of the colonies grown on this culture medium.

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Technique

The water sample is filtered trough a membrane filter of 0.45 µm 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 ± 2ºC. If in 18 h there is growth of red or colourless colonies, extend the incubation until 24 h to include late reactions of ß-galactosidase or ß-glucuronidase. Count ß-galactosidase positive colonies and ß-glucuronidase negative colonies (all colonies coloured from salmon-rose to red) as Coliform bacteria not-E. coli.
Count ß-galactosidase positive colonies and ß-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).

Supplement:

Coliform CV Selective Supplement
Vial Contents:
Necessary amount for 500 mL of complete medium.
Cefsulodin..... 2.50 mg
Vancomycin.....2.50 mg

Distilled water (Solvent)

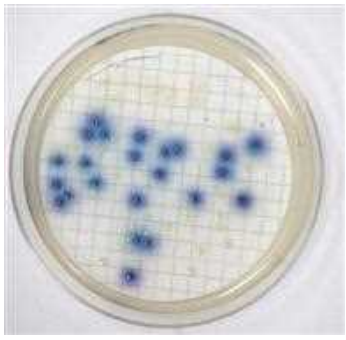
Quality control

Incubation temperature: 36ºC ±2.0 **Incubation time:** 18-24 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity)/ 10^4-10^6 CFU (Selectivity) / ≥ 10^3 CFU (specificity) according to ISO 11133:2014.

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC® 10145	Inhibited (w. inh. supplement)	w/o supplem. Inh. : Colorless colonies. Indol (-)
Escherichia coli ATCC® 25922	Productivity > 0.70	Blue-violet colonies. Indol (+)
Escherichia coli ATCC® 8739	Productivity > 0.70	Blue-violet colonies. Indol (+)
Enterobacter aerogenes ATCC® 13048	Productivity > 0.70	Salmon to red colonies. Indol (-)
Salmonella typhimurium ATCC® 14028	Good	Colorless colonies. Indol (-)
Citrobacter freundii ATCC® 43864	Productivity > 0.70	Salmon to red colonies. Indol (-)
Enterococcus faecalis ATCC® 19433	Inhibited (w. Inh. supplm.)	-


Salmonella typhimurium ATCC 14028


Salmonella typhimurium ATCC 14028
Escherichia coli ATCC® 8739


Escherichia coli ATCC 25922

References

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· ISO 7704 Standard (1985) Water Quality - Evaluation of membrane filters used for micropbiological analyses.

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).