Also known as

CCA; ACC

Specification

Selective and differential medium for the detection of coliforms and *E. coli* in waters with low bacterial background flora by MF technique.

Formula * in g/L

Casein peptone	1.00
Yeast extract	2.00
Sodium chloride	5.00
Di-sodium hydrogen phosphate	2.70
Sodium dihydrogen phosphate dihydrate	2.20
Tryptophan	1.00
Sodium pyruvate	1.00
Tergitol®7	0.15
Sorbitol	1.00
6-Chloro-3-indoxyl-ß-D-galactopyranoside	0.20
5-Bromo-4-chloro-3-indoxyl-ß-D-glucuronic acid	0.10
IPTG	0.10
Agar	13.00

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 one liter of distilled water and boil to dissolve the agar. Distributed in suitable containers and kept in a water bath of boiling water or vapor flow. Stirring frequently until complete dissolution (about 35 min). If necessary adjust the pH to 6.8 ± 0.2 (25 ° C) after treatment. No autoclave or overheat.

Description

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. The culture medium was formulated without antibiotics for water samples with low bacterial background flora, with less than 100 CFU per MF. These may be drinking water, disinfected pool water or finished water from treatment plants.
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. 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 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.
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.

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 à 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 100 millilitre (CFU /100 mL).

Quality control

Incubation temperature: 36ºC ±2.0 **Incubation time:** 21-24 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity). 10^4 CFU (Selectivity), ≥ 10^3 CFU (specificity) MF Method. according to ISO 11133:2014/Amd 1:2018

Microorganism	Growth	Remarks
Escherichia coli ATCC® 8739	Productivity > 0.70	Blue-violet colonies. Indol (+)
Escherichia coli ATCC® 25922	Productivity > 0.70	Blue-violet colonies. Indol (+)
Citrobacter freundii ATCC® 43864	Productivity > 0.70	Salmon to red colonies. Indol (-)
Pseudomonas aeruginosa ATCC® 10145	Good	Colorless colonies.
Enterococcus faecalis ATCC® 19433	Total or partial inhibited	Spiral methods / MF
Enterobacter aerogenes ATCC® 13048	Productivity > 0.70	Salmon to red colonies.

References

· ADAMS, M.., R.GRUBB, S.M. HAMER & A. CLIFFORD (1990) Colorimetric enumeration of Escherichia coli based on ß-glucuronidase activity. Appl. Environ. Microbiol. 56:2021.
· ISO 7704 Standard (1985) Water Quality - Evaluation of membrane filters used for micropbiological analyses.
· ISO 9308-1: 2014/Amd.1:2016(E) Water quality. Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· KILIAN, M. & P. BÜLOW (1976) Rapid Diagnostic of Enterobacteriaceae. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84:245-251.
· MANAFI, M & W. KNEIFEL (1989) A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliform and E. coli in water. Zentralbl. Hyg. 189:225-234.
.TURNER, K.M., L. RESTAINO & E.W. FRAMPTON (2000) Efficacy of Chromocult Coliform Agar for coliform and Escherichia coli detection in Foods. J.Food Protect. 63(4):539-541

Storage

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