Condalab

Reference: 4721 **Technical Data Sheet**

Product: # CCA CHROMOGENIC AGAR (CCA) ISO 9308

Specification

Solid, selective and differential culture medium for the detection and enumeration of total coliform and E. coli in water samples by the membrane-filtration technique acc. to ISO.

Presentation

	Packaging Details	Shelf Life	Storage
30 Prepared Plates 55 mm Plates for filtration purposes with: 9 ± 1 ml	1 box containing: 5 plastic bags with 6 plates of 55 mm/ bag.	5 months	2-25°C

Composition

Composition (g/l):

Enzymatic digest of casein	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

Description /Technique

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. 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-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 -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 technique of inoculation used in these plates is the membrane filtration technique (MF) according to the various harmonized pharmacopoeias and applicable ISO norms. The water sample is filtered trough a membrane filter of 0,45 µm of 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 ± 2°C. 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).

Quality control

Physical/Chemical control

Color : Pale yellow pH: 6.8 ± 0.2 at 25°C

Microbiological control

Inoculate:Practical range 100±20 CFU; Min. 50 CFU (Productivity).
Microbiological control according to ISO 11133:2014/ Adm 1:2018.

Aerobiosis. Incubation at 36 ± 2°C, reading at 18-24 h

Microorganism	Growth
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥70%) Dark-blue to violet colonies
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥70%) Dark-blue to violet colonies
Citrobacter freundii ATCC® 43864, WDCM 00006	Good (≥70%) Salmon to red colonies
Ps. aeruginosa ATCC® 10145, WDCM 00024	Good - Colourless
Enterococcus faecalis ATCC® 19433, WDCM 00009	Inhibited
Enterobacter aerogenes ATCC® 13048, WDCM 00175	Good - Mauve

Sterility Control

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH
Check at 7 days after incubation in same conditions

Bibliography

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