Reference: PP10ECC **Technical Data Sheet**

Product: # E. COLI CHROMOGENIC AGAR

Specification

Selective medium for detection of the total coliforms and E. coli

Presentation

	Packaging Details	Shelf Life	Storage
30 Prepared Plates 55 mm Plates for filtration purposes with: 9 ± 1 g	1 box with 5 blisters (base of aluminium, PVDC and one cellophane bag) with 6 plates 55 mm/blister	1.5 months	8-14ºC

Composition

Composition (g/l)
Peptone.....3,00
Sodium chloride.....5,00
Sodium dihydrogen phosphate.....2,20
Disodium hydrogen phosphate.....2,70
Sodium pyruvate.....1,00
L-Tryptophan.....1,00
Sorbitol.....1,00
Tergitol® 7.....0,15
Cefsulodin.....0,005
Vancomycin.....0,005
Chromogenic ß GLU 0,20
Chromogenic Salmon GAL.....0,20
Agar.....10,0

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. 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".

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 ± 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).

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Quality control

Physical/Chemical control

Color : Pale yellow pH: 6,8±0,2

Microbiological control

Inoculate with 10-100 CFU for Growth Promotion or 1000-10000 for Selectivity

Aerobiosis. Incubation at 37 ºC, reading after 24-48 hours

Microorganism	Growth
Escherichia coli ATCC 25922	Good - Purple
Escherichia coli ATCC 11775	Good - Purple
Citrobacter freudii ATCC 8090	Good - Magenta
Salmonella enterica ATCC 13076	Good - Colourless
Pseudomonas aeruginosa ATCC 9027	Inhibited
Staphylococcus aureus ATCC 6538	Inhibited
Enterococcus faecalis ATCC 29212	Inhibited

Sterility Control

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

Bibliography

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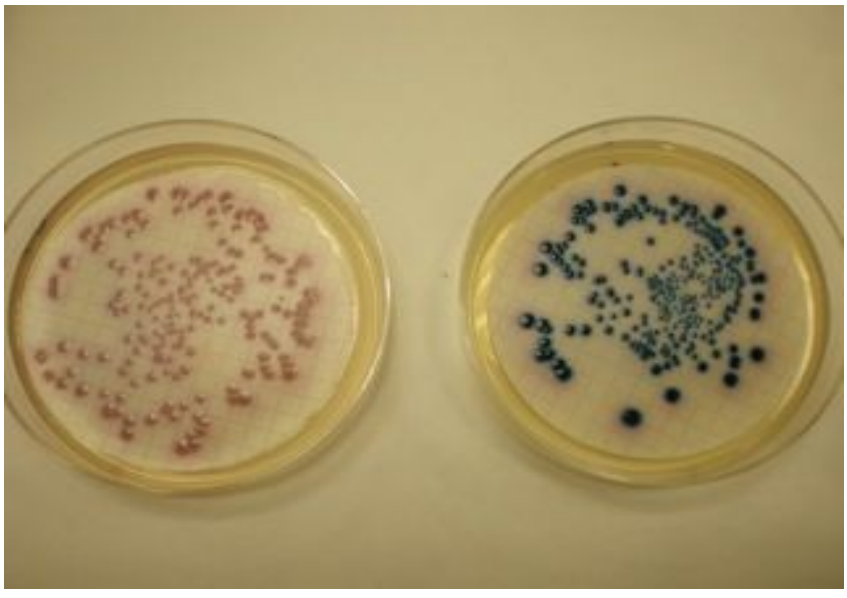

Fig 1. E. coli (dark blue) and coliforms (salmon)

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