HiCrome Chromogenic Coliform Agar (CCA)

M1991I

HiCromeChromogenic Coliform Agar is recommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.

Composition**

Ingredients	Gms / Litre
Enzymatic digest of casein	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, 2H2O	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexamine ammonium salt, monohydrate	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.92 grams(the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiChromogenic Coliform Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water samples (1). The medium contains three chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxyl-β-D-galactopyranoside to form pink to red coloured colonies (3). The enzyme ß-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxyl-β-D-glucuronic acid (2). Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Enzymatic digest of casein, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sublethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (3).

The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli*, add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Light yellow coloured opalescent gel forms in Petri plates

Reaction
Reaction of 3.09% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 34-38°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony*
Cultural Response				
Citrobacter freundii ATCC 8090	50-100	luxuriant	>=70 %	pink to red
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	>=70%	pink to red
Escherichia coli ATCC 25922	50-100	luxuriant	>=70%	dark blue to violet
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=70%	colourless

Key * : either on plate or membrane

Storage and Shelf Life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1.International Organization for Standardization. Water quality: Enumeration of ! E.coli @ and coliform bacteria. Part I-Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2.Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
3.Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.

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