

E.COLI COLIFORMS CHROMOGENIC AGAR (CCA) ISO 9308-1

CAT N°: 2080

Selective medium for the simultaneous detection of *E.coli* and other coliforms in water samples

FORMULA IN g/l

Sodium Chloride	5.00	Tryptophan	1.00
Di-sodium hydrogen phosphate	2.70	Salmon-beta-D-galactoside	0.20
Sodium dihydrogen phosphate x 2H ₂ O	2.20	Tergitol® 15-S-7 surfactant	0.15
Yeast Extract	2.00	X-beta-G-glucuronide CHX salt	0.10
Enzymatic Digest of Casein	1.00	IPTG	0.10
Sodium Pyruvate	1.00	Agar	10.00
Sorbitol	1.00		

Final pH 6.8 ± 0.2 at 25°C

PREPARATION

Suspend 26.45 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45-50°C and pour into petri plates. Homogenize gently and dispense into Petri dishes. The prepared medium should be stored at 8-15°C, protected from light. The color of the prepared medium is amber.

The standard recommends not using freshly prepared plates but to store them at 5±3°C, away from light, during at least one month. Dry off moisture before use.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

E.COLI-COLIFORMS CHROMOGENIC AGAR (CCA) is a selective medium for the detection of *E.coli* and other coliforms in water and foods.

The interaction of ingredients in the medium, such as peptone, sorbitol, etc., allows a quick colony growth, including infectious Coliforms. Tergitol inhibits Gram-positive bacteria. Sodium chloride maintains the osmotic balance and Phosphate salts act as a buffer system. Bacteriological agar is the solidifying agent. The chromogenic mixture contains chromogenic substrates such as Salmon-GAL and X-glucuronide. Coliform enzymes produced, such as galactosidase and glucuronidase, cleave these substrates, resulting in the different coloration of certain bacteria colonies. The β-D-galactosidase cleaves Salmon-GAL substrate, and gives a salmon to red color to the coliform colonies. *E.coli* cleaves both substrates Salmon-Gal and X-glucuronide, giving a dark blue to violet color to the colonies, easily distinguishable from other coliform colonies that have a salmon to red color. The addition of tryptophan to the medium allows the performance of the Indole test for further *E. coli* confirmation.

Process

- 1.- Filter sample through a membrane
- 2.- Place the membrane filter over a coliforms chromogenic agar plate.
- 3.- Incubate the membrane filter at (36±2)°C during (21±3) hrs.
- 4.- Count the β-D-galactosidase colonies (pink to red in color) as presumptive coliform bacteria that are not *E. coli*
- 5.- To avoid false positive results, caused by oxidase-positive bacteria, for example *Aeromonas spp*, confirm bacterial colonies through an oxidase-negative reaction.

The positive colonies β-D-galactosidase and β-D-glucuronidase (dark blue to violet) are counted as *E. coli*.

The total coliform bacteria count is the sum of oxidase-negative colonies, β -D-galactosidase-positive colonies (pink to red) and all colonies which dark blue to violet.

Note: Some *Shigella* strains contain the enzyme β -D-glucuronidase and can grow as light blue colonies. The negative *E. coli* β -glucuronidase colonies are colorless, e.g. *E. coli* O157:H7.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of $36 \pm 2^\circ\text{C}$ and observed after 21 ± 3 hours.

Microorganisms	Quantitative Productivity	Qualitative Selectivity	Qualitative Specificity	Colony Color
<i>Escherichia coli</i> ATCC 25922	$\geq 0,7$			Blue-dark violet
<i>Escherichia coli</i> ATCC 8739	$\geq 0,7$			Blue-dark violet
<i>Enterobacter aerogenes</i> ATCC 13048	$\geq 0,7$			Red- rose
<i>Enterococcus faecalis</i> ATCC 19433		Total inhibition		
<i>Pseudomonas aeruginosa</i> ATCC 10145	Growth		Colorless	Colorless

BIBLIOGRAPHY

ISO 9308-1/2014 Water quality — Enumeration of *Escherichia coli* and coliform bacteria —Part 1: Membrane filtration method for waters with low bacterial background flora.

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STORAGE

Once opened keep powdered medium closed to avoid hydration.

