Chromogenic Coliforms Agar (CCA) ISO

Cat. 2080

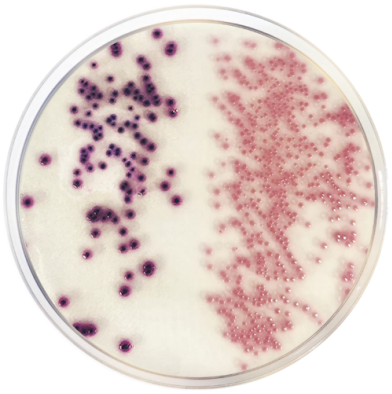
Selective medium for the simultaneous detection of E. coli and other coliforms in water samples.

Practical information

Aplications	Categories
Selective enumeration	Coliforms
Selective enumeration	Escherichia coli

Industry: Water

Regulations: ISO 11133 / ISO 9308



Principles and uses

Chromogenic Coliforms Agar (CCA) is a selective medium for the detection of E. coli and other coliforms in waters and foods. The recovery and enumeration of Escherichia coli and coliforms are important indicators of environmental and food hygiene.

The interaction of ingredients in the medium, such as peptone, sorbitol and pyruvate, grants a quick colony growth, including infectious coliforms and also permits the recovery of sublethal thermally injured coliforms. Tergitol-7 inhibits Gram-positive bacteria and some Gram-negative without affecting the coliform bacteria. Sodium chloride maintains the osmotic balance and phosphate salts act as a buffer system. Bacteriological agar is the solidifying agent.

Detection of ß-glucuronidase is widely used to differentiate Escherichia coli, as the enzyme is present in E. coli but not in other member of coliform group. The chromogenic mixture contains chromogenic substrates: Salmon-GAL and X-glucuronide. Coliform enzymes produced, ß-D-galactosidase and ß-D-glucuronidase, cleave these substrates resulting in the different coloration of bacteria colonies. The ß-D-galactosidase cleaves Salmon-GAL substrate, and gives a salmon-red color to the coliform colonies. The ß-D-glucuronidase, enzyme characteristic of E. coli, cleaves X-glucuronide, giving a blue color to these colonies. 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 addition of tryptophan to the medium allows the performance of the Indole test for further E. coli confirmation.

Formula in g/L

Enzymatic digest of casein	1	Bacteriological agar	10
IPTG	0,1	Salmon-beta-D-Galactoside	0,2
Sodium chloride	5	Sodium pyruvate	1
Sorbitol	1	Tergitol® 15-S-7 surfactant	0,15
Tryptophan	1	X-beta-G-glucuronide CHX salt	0,1
Yeast extract	2	Sodium dihydrogen phosphate x 2H2O	2,2
Di-sodium hydrogen phosphate	2,7		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

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 ºC, homogenize gently and dispense into Petri dishes.

Instructions for use

For the enumeration of E. coli and coliform bacteria according to ISO 9308:

- Filter sample through a membrane .
- Place the membrane filter over a E. Coli Coliforms Chromogenic Agar plate.
- Invert Petri dish and incubate at 36±2 ºC during 21±3 h.
- Count the ß-D-galactosidase colonies (pink to red in color) as presumptive coliform bacterias that are not E. coli
-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 ß-D-galactosidase and ß-D-glucuronidase (dark blue to violet) are counted as E. coli.
- The total coliform bacteria count is the sum of oxidase-negative colonies, ß-D-galactosidase-positive colonies (pink to red) and all colonies which dark blue to violet.
- Some Shigella strains contain the enzyme ß-D-glucuronidase and can grow as light blue colonies.
- The negative E. coli ß-glucuronidase colonies are colorless, e.g. E. coli O157:H7.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber	6,8±0,2

Microbiological test

According to ISO 11133:
Incubation conditions: (36±2 ºC / 21±3 h).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).
Reference media: TSA.

Microorganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 10145	Growth	Colorless colonies
Klebsiella aerogenes ATCC 13048	Good growth >70%	Red to pink colonies
Enterococcus faecalis ATCC 19433	Total inhibition	
Escherichia coli ATCC 25922	Good growth >70%	Dark blue to violet colonies
Escherichia coli ATCC 8739	Good growth >70%	Dark blue to violet colonies

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

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