E. coli-Coliforms Chromogenic Agar Base (BOE)

Cat. 1491

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

Practical information

Aplications	Categories
Detection	Coliforms
Detection	Escherichia coli

Industry: Water / Food

Principles and uses

E. coli-Coliforms Chromogenic Agar Base 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. Selectivity is enhanced by the Cefsulodine and Vancomycin, supplied by the Supplement E. coli-Coliformes (Cat. 6041), act against pseudomonas and Gram negative, oxidase positive bacteria, enterococci and other Gram positive 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.

Note: Some Shigella strains contains the enzyme ß-D-glucuronidase and can grow aslight blue colonies. The negative E. coli b-ß-glucuronidase colonies are Salmon, e.g. E. coli O157:H7.

Formula in g/L

Bacteriological agar	10	Casein peptone	3
Sodium chloride	5	Sodium dihidrogenphosphate	2,2
Sodium pyruvate	1	Sorbitol	1
Tryptophan	1	Di-sodium hydrogen phosphate	2,7
Salmon GAL	0,2	X-Glucuronide	0,2
Tergitol 7	0,15		

Preparation

Suspend 13,25 grams of the medium in 500 ml 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 and aseptically add one vial of the E.coli-coliforms Supplement (Cat. 6041) reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes.

Instructions for use

The following techniques may be used:

Spread plate method (Digralsky):
- In a Petri dish, add 12-15 ml of molten agar and let it solidify.
- Inoculate 0,1 ml of the initial suspension and/or diluted sample.
- Extend the inoculum with a sterile loop on the agar surface.
- Incubate the plates in an inverted position at a temperature of 36±2 ºC for 18-24 hours.

Poured plate method:
- Deposit 1 ml of the initial suspension and/or diluted sample in an empty Petri dish.
- Add 12-15 ml of agar cooled to 45 ºC in each Petri dish and mix gently moving the plate.
- Allow the plates to solidify and incubate in an inverted position at a temperature of 36±2 ºC for 18-24 hours.

Filter membrane method:
- Dry the surface of the prepared plates.
- Filter an appropriate volume of the sample through the membrane.
- Place the membrane on the surface of the agar plate, avoiding the formation of air bubbles.
- Invert the plates and incubate at 36±2 ºC for 18-24 hours.

Incubate until 24 hours to observe possible retarded ß-Galactosidase and ß-Glucurinidase reactions.

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

Incubation conditions: (36±2 ºC / 18-24 h).

Microrganisms	Specification	Characteristic reaction
Salmonella enteritidis ATCC 13076	Good growth	Colorless colonies
Enterococcus faecalis ATCC 19433	Total inhibition	
Escherichia coli ATCC 25922	Good growth	Blue-dark violet colonies
Citrobacter freundii ATCC 8090	Good growth	Salmon colonies
Escherichia coli ATCC 8739	Good growth	Blue-dark violet colonies

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Alonso, J.L. Soriano, K., Amoros I., Ferrus, M.A. 1998 Cevartitatine determination of E. coli and fecal coliforms in water using a chromogenic medium. BOLETÍN OFICIAL DEL ESTADO. Num. 78 Martes 31 de marzo de 2009 Sec. I. Pág. 30417. Orden SCO/778/2009, de 17 de marzo, sobre métodos alternativos para el análisis microbiológico del agua de consumo humano.