Rapid HiColiform Agar

M1465

Rapid HiColiform Agar is used for detection and confirmation of *Escherichia coli* and total coliforms on the basis of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrates.

Composition**

Ingredients	Gms / Litre
Peptone, special	5.000
Sodium chloride	5.000
Sorbitol	1.000
Dipotassium hydrogen phosphate	2.700
Potassium dihydrogen phosphate	2.000
Sodium lauryl sulphate	0.100
Chromogenic substrate	0.080
Fluorogenic substrate	0.050
IPTG(1-Isopropyl-β-D-1-thiogalactopyranoside)	0.100
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.03 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

The Rapid HiColiform Agar is modification of LMX Broth described by Manafi and Kneifel (2). Rapid HiColiform Agar is used for the simultaneous detection of total coliforms and *Escherichia coli* . This media is useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorogenic substrates (1-6).

Special peptone which is rich in tryptophan content, provides essential growth nutrients and is useful for the simultaneous detection of indole production. Sorbitol provides the carbon source. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms. The fluorogenic substrate, is split by enzyme ß-D-glucuronidase, which is specifically found in *Escherichia coli* . The reaction is indicated by a blue fluorescence under UV light . The presence of total coliforms is indicated by a blue-green colour of the colonies due to the cleavages of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of ß-D-galactosidase. To confirm presence of *E. coli* , add 2-3 drops of Kovacs reagent over the suspected colony. The colony turns red within 2 minutes incase of 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, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response

M1465: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Colour of medium/ Colony	Fluorescence (under uv)	Indole production
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	blue-green	negative reaction	negative reaction
Escherichia coli ATCC 25922	50-100	luxuriant	blue-green	positive reaction	positive reaction
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	blue-green	negative reaction	negative reaction
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	yellow	negative reaction	negative reaction

Storage and Shelf Life

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

Reference

1.Hahn G. and Wittrock E., (1991), Acta Microbiologica Hungarica 38(3-4):265-271.
2.Manafi. M. and Kneifel W., (1989), Zbl. Hygiene and Umweltmedizin 189:225-234.
3.Manafi M., (1990), Forum Stadte-Hygiene 41:181-184.
4.Manafi M., (1991), Ernahrung / Nutrition, 15, Nr. 10.
5.Manafi M. and Kneifel W., (1991), Acta Microbiologica Hungarica 33(3-4):293-304.
6.,,Manafi M., Kneifel B. and Bascon S., (1991), Microbiol. Rev., 55:335-348.

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