Rapid HiColiform™ Broth

M1453

Intended use

Recommended 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	g / L
Special peptone	5.000
Sodium chloride	5.000
Sorbitol	1.000
Dipotassium hydrogen phosphate	2.700
Potassium dihydrogen phosphate	2.000
Sodium lauryl sulphate (SLS)	0.100
Chromogenic substrate	0.080
Fluorogenic substrate	0.050
IPTG	0.100
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16.03 grams in 1000 ml purified / distilled water. For double strength broth use 32.06 grams of M1453 in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense as desired.

Principle And Interpretation

Rapid HiColiform™ Broth is a modification of LMX Broth described by Manafi and Kneifel (1). This medium is useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorognic substrates (1-8).

The fluorogenic substrate is split by enzyme ß-D-glucuronidase specifically found in *Escherichia coli*. The reaction is indicated by the development of a blue fluorescence under UV light. The presence of total coliforms is indicated by blue-green colourations due to the cleavage of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of ß-D-galactosidase. To confirm presence of *E.coli* overlay the medium with Kovacs reagent. The layer turns red within 2 minutes in case of positive reaction.

Special peptone serves as a source of carbon and nitrogen compounds, long chain amino acids, vitamins and other essential growth nutrients. Sorbitol is the fermentable carbohydrate. 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.

Type of specimen

Food samples; Water samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(10). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. 97% of the *E.coli* strains are ß-D-glucuronidase positive, however few strains may show negative fluorescence
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

3.Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution having slight precipitate in tubes

Reaction

Reaction of 1.60% 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 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Colour of Medium	Fluorescence (under uv)	Indole reaction
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	blue-green	negative	negative reaction
Escherichia coli ATCC 25922 (00012*)	50-100	luxuriant	blue-green	positive	positive reaction

Key : * Corresponding WDCM numbers. # Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Hahn G. and Wittrock E., (1991), Acta Microbiologica Hungarica 38(3-4):265-271.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Manafi. M. and Kneifel W., (1989), Zbl. Hygiene and Umweltmedizin ,189:225-234.
5. Manafi M., (1990), Forum Stadte-Hygiene 41:181-184.
6. Manafi M., (1991), Ernahrung / Nutrition, 15, Nr. 10.
7. Manafi M. and Kneifel W., (1991), Acta Microbiologica Hungarica 33(3-4):293-304.
8. Manafi M., Kneifel B. and Bascon S., (1991), Microbiol. Rev., 55:335-348.
9. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 03/ 2024

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