HiCrome™ m-Coliconfirm Broth Basee

M2064

Intended use

Recommended for detection of *E.coli* and other total coliforms in water samples by membrane filtration.

Composition**

Ingredients	g / L
Tryptone	8.000
Yeast extract	0.500
Lactose	0.600
Sodium chloride	3.000
Dipotassium hydrogen phosphate	1.750
Potassium dihydrogen phosphate	1.250
Sodium pyruvate	1.000
Octyphenol ethoxylate	0.500
Magnesium sulphate	0.300
Sodium azide	0.020
L-Methionine	0.100
Methylene blue	0.016
Cyclohexylammonium salt	0.200
Chromogenic mixture	0.200
Final pH (at 25°C)	7.00±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.43 gram in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Aseptically add the rehydrated contents of ECC Selective Supplement (FD344) and 10ml of TTC Solution, 1% (FD057). Mix well and aseptically add desired quantity (2 to 5 ml) of broth on sterile absorbent cotton pad or sterile filter paper for saturation. The nutrient pad should be used within 24 hours of saturation.

Principle And Interpretation

This is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water (1). The water sample is filtered through membranes and then placed on pad saturated with medium and incubated at 35 ±5°C for 24 hours in sealed Petri plates.

Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential nutrients. Yeast extract serves as a source of vitamins. Lactose is the fermentable carbohydrate. The phosphates in the medium buffers the medium. Sodium chloride maintains the osmotic balance. The enzyme beta-glucuronidase produced by *E.coli* utilizes the chromogenic substrate to produce blue-purple coloured colonies. Coliforms other than *Escherichia coli* turn red as they reduce TTC (2,3,5-triphenyl tetrazolium chloride). Thus, the resulting colour distinction allows simple interpretation of test without further confirmation. Methylene blue and ECC selective supplement containing imparts selectivity to the medium. Non-coliforms usually give white coloured colonies.

Type of specimen

Water samples.

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).

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.Overgrowth of non-coliform organisms may interfere with the total coliform organisms.
2.ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
3.Since the medium is highly selective, some strains may show poor growth due to nutritional variations.
4. 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

Cream, clear to slightly opalescent solution,may have slight precipitate.

Reaction

Reaction of 1.74% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 34.5-35.5°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Colour of colony on membrane
Citrobacter freundii ATCC 8090	50-100	luxuriant	red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	blue
Escherichia coli ATCC 35218	50-100	luxuriant	blue
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	-
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	red
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	-
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	-

Key : (*) Corresponding WDCM numbers

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. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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.

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