

Chromogenic Coliform Medium

MF034/F▽

Intended Use

Recommended for detection of *E.coli* and coliforms in water samples.

Directions

The test sample should be filtered through a sterile membrane filter having pore size of 0.22μ / 0.45μ. Rehydrate the nutrient pad with 2.0-2.5 ml sterile distilled / purified water. After filtration, remove the membrane filter aseptically using sterile forceps. Place the membrane filter on rehydrated nutrient pad. Incubate the inoculated nutrient. Interpret the results qualitatively by observing the presence or absence of growth and quantitatively by counting the number of colonies on the surface of the membrane filter and calculating CFU/ml.

Principle And Interpretation

DriFilter Membrane Nutrient Pad Medium is ready to use sterile culture media in the form of a 50 mm biological inert absorbent pads impregnated with Sabouraud Dextrose Medium, then dried and sterilized in 55 mm petri plate. They eliminate the need of laborious media preparation and autoclaving procedures. The nutrient pads are to be just rewetted with sterile distilled water and are ready to use. Use of nutrient pads allows larger sample volumes to be tested at a time. Interpretation of results is directly by counting the CFUs and also quantifies the microbial load present in the sample. HiCrome™ Chromogenic Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water samples (4). The medium contains three chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxy 1-β-D-galactopyranoside to form pink to red coloured colonies (2). The enzyme β-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxy 1-β-D-glucuronic acid (2) Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Tryptone, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sub-lethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (2). The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli*, add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples - Water and wastewater

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. The media should be handled by trained personnel only. 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. Further biochemical testing is required for identification of microorganism.
2. Certain variations in colour may be observed.

Quality Control

Appearance

Dry filter membrane pad of 50mm diameter

Colour

Pale coloured nutrient pad

Sterility test

Passes release criteria

Cultural Response

Cultural characteristics observed after incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Colour of Colony
<i>Escherichia coli</i> ATCC 25922 (00013)*	50-100	luxuriant	dark blue to violet
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	Pink to Red
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	$\geq 10^4$	inhibited	
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	Pink to Red
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	luxuriant	colourless

Key * : Corresponding WDCM numbers

Formerly known as *Enterobacter aerogenes*.

Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label.

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 (3,4).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part I-Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
6. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.

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Note:

MF000 - Sterile pad packed individually in sterile Petri plate without Membrane Filter

MF000F[▽] - Sterile pad packed individually in sterile Petri plate with sterile Membrane Filter (0.45 mm).

Disclaimer :

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