Technical Data

R2A Medium (Economy pack) (without Membrane Filter)

MF030E

Intended Use:

Recommended for heterotrophic plate count of treated potable water using longer incubation periods.

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 R2A Medium, then dried and sterilized in sterile disposable plastic bag. 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. The heterotrophic plate count (HPC), formerly known as the standard plate count is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment, in distribution systems or in swimming pools. R-2A Agar is recommended by APHA (1, 6) for estimating the heterotrophic plate count by the pour plate, spread plate or membrane filter procedure. R-2A Agar is formulated as per Reasoner and Geldreich (5). Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former (2). Therefore the use of a low nutrient medium like R-2A Agar incubated for longer incubation periods allows these stressed organisms to grow well.

Many bacteria from natural waters which contain limited nutrients at ambient temperature, grow best on the media with less nutrient levels. They grow better at the temperatures below the routine laboratory incubation temperatures of 35 to 37°C (2). Acicase, proteose peptone and yeast extract provide nitrogen, carbon compounds, vitamins, amino acids and minerals. Dextrose/ glucose serves as an energy source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Magnesium sulphate is a source of divalent cations and sulphate. Dipotassium hydrogen phosphate is used to balance the pH of the medium. The number of colonies on a plate are reported as CFU (Colony Forming Units) per volume of sample.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standard (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. Longer incubation time other than specified is required for slow growing microorganisms.

2. The media is intended for water samples for recovery of stressed or injured organisms.

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

Dry filter membrane pad of 50mm diameter

Colour

Pale coloured nutrient pad

Sterility test

Passes release criteria

Cultural Response

Cultural characteristics observed *by using standard ATCC cultures after an incubation for 24 - 72 hours at 35-37°C.

Organism	Inoculum (CFU)	Growth	Color of colony
Escherichia coli ATCC 25922 (00013*)	50-100 50-	good-luxuriant	Red - maroon
Enterococcus faecalis ATCC 29212 (00087*)	100 50-100	good-luxuriant	Red - maroon
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	Red - maroon
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	Red - maroon
Salmonella Typhi ATCC 6539		good-luxuriant	Red - maroon

Key : * Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 10-30°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, Wastewater, 20th Ed., American Public Health Association, Washington, D.C.

2. Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.

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. 1.66

5. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.

6. 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.

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