

R-2A Agar Plate

MP962

For heterotrophic plate count of treated potable water, using longer incubation period.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate	0.500
Yeast extract	0.500
Proteose peptone	0.500
Dextrose	0.500
Starch, soluble	0.500
Dipotassium phosphate	0.300
Magnesium sulphate	0.024
Sodium pyruvate	0.300
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate

Principle And Interpretation

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, 2) 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 (3). Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former (4). 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 (4). Casein acid hydrolysate, proteose peptone and yeast extract provide nitrogen, vitamins, amino acids, carbon and minerals. Dextrose 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 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.

Quality Control

Appearance

Sterile R-2A Agar in 90mm disposable plates.

Colour

Light yellow coloured medium

Quantity of medium

25ml of medium in disposable plate

Reaction

7.00- 7.40

Cultural response

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

Sterility test

Passes release criteria

Cultural Response

Organism	Growth
Cultural response	
<i>Escherichia coli</i> ATCC 25922	Good-luxuriant
<i>Enterococcus faecalis</i> ATCC 29212	Good-luxuriant
<i>Candida albicans</i> ATCC 10231	Good-luxuriant
<i>S. serotype Enteritidis</i> ATCC 13076	Good-luxuriant
<i>S. serotype Typhi</i> ATCC 6539	Good-luxuriant

Storage and Shelf Life

Store between 15-25°C. Use before expiry date on the label.

Reference

1. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
2. Downes F. P. and Ito K., (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
3. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol.,49:1.
4. Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.

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