Technical Data

R2A Agar

SMEB962D

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

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

R2A Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized; hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium as desired and allowed to solidify.

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 glass bottle conataining slightly opalescent R2A Agar.

Colour
Light Amber coloured medium

Quantity of medium
500 ml ml of medium in glass bottle

Reaction
7.00- 7.40

Sterility test
Passes release criteria

Cultural response
Cultural characteristics after melting the medium and pouring into sterile petri plates. The plates are inoculated with following test organisms and incubation at 35 -37°C for 48-72 hours.

Organism	Growth	Inoculum (CFU)	Observed Lot value (CFU)	Recovery
Candida albicans ATCC 10231	good-luxuriant	50 -100	25 -100	>=70 %
Enterococcus faecalis ATCC 29212	good-luxuriant	50 -100	25 -100	>=70 %
Salmonella Enteritidis ATCC 13076	good-luxuriant	50 -100	25 -100	>=70 %
Salmonella Typhi ATCC 6539	good-luxuriant	50 -100	25 -100	>=70 %
Escherichia coli ATCC 8739	good-luxuriant	50 -100	25 -100	>=70 %

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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