Dehydrated Culture Media
Bases / Media Supplements

Technical Information

R-2A Agar

Product Code: DM 1962

Application: - R-2 A Agar is used for heterotrophic plate count of water samples 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
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & 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 monitoring changes during water treatment, in distribution systems of water or in swimming pools. R-2A Agar is also 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 by Reasoner and Geldreich [3]. Stressed or injured organisms during water treatment are unable to grow on high nutrient media, because the faster growing organisms outgrow the former [4. 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 numbers of colonies on a plate are reported as CFU (Colony Forming Units) per volume of sample.

Methodology

Suspend 18.12 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. DO NOT OVERHEAT. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 1.8 1% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range:-
7.00-7.40

Cultural Response/Characteristics
DM 1962: Cultural characteristics observed *by using standard ATCC cultures after an incubation at 35-37°C for 24-72 hours.(*-In case of water samples from fields it is suggested to incubate further for upto 7 days to ascertain the absence of organisms)

Organism	Inoculum (CFU)	Growth	Recovery
Candida albi cans ATCC 10231	50-100	good-luxuriant	>=50%
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=50%
Salmonella Enteritidis ATCC 13076	50-100	good-luxuriant	>=50%
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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.

Disclaimer :

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