TM 269 – R-2A AGAR

INTENDED USE

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

PRODUCT SUMMARY AND EXPLANATION

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 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. Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former. 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.

COMPOSITION

Ingredients	Gms / Ltr
Casein Acid Hydrolysate	0.500
Yeast extract	0.500
Proteose peptone	0.500
Dextrose (Glucose)	0.500
Starch soluble	0.500
Dipotassium hydrogen phosphate	0.300
Magnesium sulphate	0.024
Sodium pyruvate	0.300
Agar	15.000

PRINCIPLE

This medium consists of Casein acid hydrolysate, proteose peptone and yeast extract which 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.

INSTRUCTION FOR USE

- Dissolve 18.12 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 min. DO NOT OVERHEAT. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light yellow coloured clear to slightly opalescent gel forms in petri plates.
pH (at 25°C) : 7.2 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation. (In case of water samples from fields it is suggested to incubate further for upto 7 days to ascertain the absence of organisms).

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Candida albicans	10231	10-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Escherichia coli	25922	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Salmonella Enteritidis	13076	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Pseudomonas aeruginosa	9027	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Staphylococcus aureus subsp. aureus	6538	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Bacillus subtilis subsp. spizizenii	6633	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Aspergillus brasiliensis	16404	10-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours
Enterococcus faecalis	29212	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours

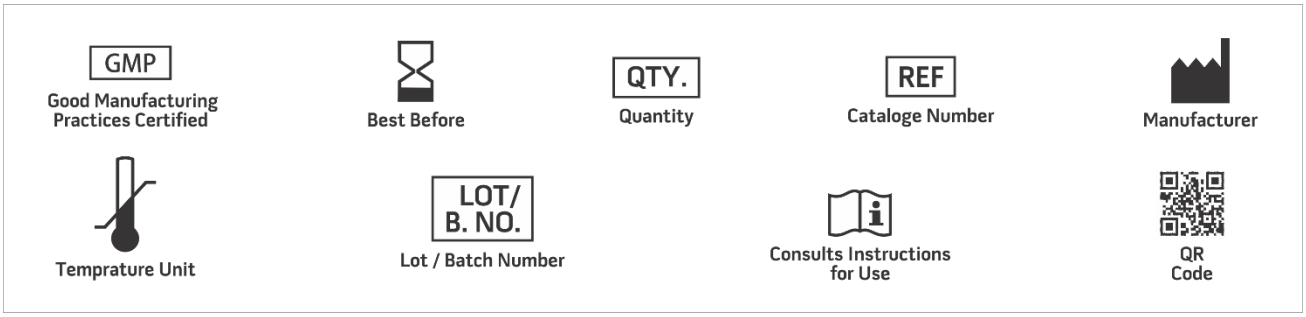
Salmonella Typhi	6539	50-100	Good-luxuriant	>=50%	30-35°C	24-72 Hours

PACKAGING:
In pack size of 100 gm and 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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. 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.
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.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
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