Technical Information

R2A Agar, Modified

Product Code: DM 2743

Application: - R2A Agar, Modified is used for the enumeration and cultivation of bacteria from potable water.

Composition**

Ingredients	Gms / Litre
Casein Enzymic hydrolysate	0.250
Peptic digest of animal tissue	0.250
Casein Acid hydrolysate	0.500
Yeast extract	0.500
Glucose	0.500
Starch soluble	0.500
Dipotassium phosphate	0.030
Magnesium sulphate, heptahydrate	0.500
Sodium pyruvate	0.030
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

R2A Agar, Modified is recommended in standard methods for pour plate, spread plate and membrane filter methods for hetrotrophic plate count (1). It was developed by Reasoner and Geldreich (2) for bacterial plate counts of treated potable water. The HPC, heterotrophic plate count formerly known as the standard plate count is a procedure for estimating the number of live bacteria in water and measuring changes during water treatment in distribution systems or in swimming pools. The use of low nutrient media favours growth of injured or stressed organisms at longer incubation periods as compared to the use of high nutrient media. As compared to Tryptone Glucose Agar or Standard methods agar, R2A agar has been reported to give improved recovery of stress and chlorine tolerant bacteria from drinking water systems (3,4,5).

Enzymic digest of casein, enzymatic digest of animal tissue, casein acid hydrolysate and yeast extract supplies necessary nitrogen sources, carbohydrates, vitamins, minerals and growth factors to growing organisms. Dextrose acts as carbon source, Soluble starch aids in recovery of injured organisms toxic metabolic byproducts while sodium pyruvate increases recovery of stressed cells. Magnesium sulphate acts as a source of divalent cations and sulphate. Dipotassium phosphate is used to balance the pH of medium. Agar acts as a solidifying agent.

For heterotrophic plate count, one can prepare set of dilutions of the test sample. Either of the methods; spread plate pour plate or membrane filter method can be used for isolation. However pour plate method is not much practiced as the recovery of stressed bacteria may be compromised by heat shock (44-46°C) and low oxygen tension that are part of procedure (6,7). In case of spread or pour plate maximum of 1 ml sample should be used. Please note that the volume of sample for membrane filtration technique varies from spread or pour plate. While studying the sample incubate the plates at 35-37°C for minimum 72 hours whereas 5 days when at 20 to 28°C. The optimum incubation time should be 5-7 days in either case. Results may be recorded as colony forming units per ml. At times incubation of longer period is also required to recover additional slow growing bacteria. The number of colonies obtained on a plate are reported as CFU per volume of sample.

Methodology

Suspend 18.12 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 1.81% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range

7.00-7.40

Cultural Response

DM 2743: 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 up to 7 days to ascertain the absence of organisms)

Organism	Inoculum (CFU)	Growth	Recovery
Candida albicans ATCC10231	50-100	good-luxuriant	>=50%
Enterococcus faecalis ATCC29212	50-100	good-luxuriant	>=50%
Escherichia coli ATCC25922	50-100	good-luxuriant	>=50%
Salmonella Enteritidis ATCC13076	50-100	good-luxuriant	>=50%
Salmonella Typhi ATCC6539	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 8739	50-100	good-luxuriant	>=50%
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=50%
Escherichia coli NCTC 9002	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry date on label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Eaton, A.D., L.S.Clesceri, and A.E. Greenberg (eds.), 1995, Standard Methods for the examination of water and wastewater, 19th ed. American Public Health Association,Washington, D.C.
2. Reasoner, D. J and Geldreich, E.E ,1979, A new medium for the enumeration and subculture of bacteria form potable water. Abstracts of the Annual meeting of the American Society for microbiology 79th Meeting, Paper No. N7.
3. Fiksdal,L.,E.A. Vik, A.Mills, and T.Staley, 1982, Non-standard methods for enumerating bacteria in drinking water. Journal AWWA, 74: 313-318.

4. Kelly, A.J., C.A.Justice, and L.A. Nagy, 1983, Predominance of chlorine tolerant bacteria in drinking water systems. Abstracts of the Annual meeting of the American Society for Microbiology 79th Meeting paper No. Q122.
5. Means E.G., L. Hanami, H.F.Ridgway, and B.H. Olson, 1981, Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. Journal AWWA 53: 585-590.
6. VanSoestberger, A.A., and C.H. Lee. 1969 Appl. Microbiol. 18: 1092.
7. Klein D.A. and S. Wu. (1974). Appl. Microbiol. 27: 429.

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