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R2A Agar

Nutrient medium for the determination of the heterotrophic total bacterial count in drinking water.

General Information

R2A Agar is a medium with a low nutrient content, which, in combination with a low incubation temperature and an extended incubation time, is specially suitable for the recovery of stressed and chlorine-tolerant bacteria from drinking water.

The nutrient medium conforms with recommendations of the standard methods (US-EPA) and the European Pharmacopeia for the examination of water.

Mode of Action

The low concentration of yeast extract, casein hydrolisate, peptone and glucose allows a wide spectrum of bacteria to grow without the fast-growing bacteria suppressing the slow-growing species, such as would be the case on richly nutritious media like e.g. Plate Count Agar.

The content of starch and pyruvate allows particularly the injured bacteria to grow again more quickly.

Typical Composition (g/litre)

Yeast extract 0.5; proteose peptone 0.5; casein hydrolysate 0.5; glucose 0.5; soluble starch 0.5; sodium pyruvate 0.3; dipotassium hydrogenphosphate 0.3; magnesium sulphate anhydrous 0.024; agar-agar 15.0.

Preparation

Suspend 18.2 g in 1 litre demin. water and heat in a boiling water bath or flowing steam until the medium has completely dissolved. Autoclave for 15 min. at 121 °C, cool to 45–50 °C and pour into sterile Petridishes.

pH : 7.2 ± 0.2 at 25 °C

The prepared medium is clear to slightly opalescent and colourless.

Storage

In correct storage conditions (+2 – +8 °C, protected from light and dehydration) the plates can be stored for 4 weeks.

Experimental Procedure

The determination of the total bacterial count using R2A agar can be carried out with the pour plate, spread plate and membrane filter methods.

If an incubation time of more than 3 days is used, the plates should be protected from dehydration.

Incubation temperature	Minimum Incubation time	Maximum Incubation time
35 °C	72 hours	5-7 days
20 or 28 °C	5 days	7 days

Evaluation

The number of colonies is counted and the bacteria count/ml is calculated noting the incubation temperature and incubation period.

Literature

Eaton, A. D., L.S. Clesceri, and A.E. Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater, 19th. Ed. APHA, Washington D.C.

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.

Means, E.G., L. Hanami, H.F. Ridgway, and B.H. Olson. 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distributing systems. Journal AWWA. 53:585-590.

Reasoner, D.J., and E.E. Geldreich. 1979. A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.

Ordering Information

Product	Ordering No.	Pack size
R2A Agar	1.00416.0500	500 g

Quality control

Test strains	Incubation at 35°C for 24 h	
	Inoculum	Recovery rate
Escherichia coli ATCC 8739	10 - 100	> 70%
Pseudomonas aeruginosa ATCC 9027	10 - 100	> 70%
Pseudomonas aeruginosa ATCC 27853	10 - 100	> 70%
Staphylococcus aureus ATCC 6538	10 - 100	> 70%
Bacillus subtilis ATCC 6633	10 - 100	> 70%

Test strains	Incubation at 20 - 25°C for 72 h	
	Inoculum	Recovery rate
Escherichia coli ATCC 8739	10 - 100	> 70%
Pseudomonas aeruginosa ATCC 9027	10 - 100	> 70%
Pseudomonas aeruginosa ATCC 27853	10 - 100	> 70%
Staphylococcus aureus ATCC 6538	10 - 100	> 70%
Bacillus subtilis ATCC 6633	10 - 100	> 70%
Pseudomonas fluorescens ATCC 17386	10 - 100	> 50%
Methylobacterium extorquens NBRC 15911	10 - 100	> 50%