R2A Agar EP/USP

Cat. 1071

For the total aerobe count in treated waters.

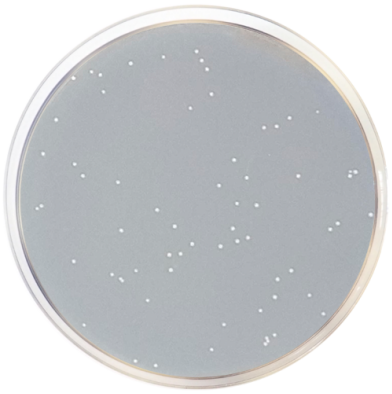
Practical information

Aplications	Categories
Non selective enumeration	Mesophilic aerobic

Industry: Water / Pharmaceutical/Veterinary / Clinical / Quality Control

Regulations: USP / European Pharmacopoeia

CE IVD



Principles and uses

R2A AGAR was developed by Reasoner and Geldreich for bacteriological plate counts of treated potable water, being able to recover the stressed chlorine-treated bacteria. Nutritionally rich mediums suppress these slow growing bacteria, whereas a low nutrient medium, such as R2A Agar, in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria.

Proteose peptone and Casein Hydrolysate provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Dextrose is a source of fermentable carbohydrate as an energy source; Starch absorbs toxic metabolic byproducts and thereby aids the recovery of injured organisms. Sodium pyruvate increases the recovery of stressed cells. Magnesium sulfate provides divalent cations and sulfate. Dipotassium phosphate is used to balance the pH and provide phosphate. Bacteriological agar is solidifying base.

R2A Agar is recommended in Standard Methods for the Examination of Water and Wastewater for pour plate, spread plate and membrane filter methods for heterotrophic counts.

The European Pharmacopoeia recommends in paragraph Water for Injections (Aqua ad iniectabilia) this medium for the microbial monitoring of this water during the process of production and storage in the preparation of medicines. It is also recommended for analysis of Water for preparations of extracts, water highly purified and water purified.

Formula in g/L

Glucose	0,5	Bacteriological agar	15
Dipotassium phosphate	0,3	Sodium pyruvate	0,3
Starch	0,5	Yeast extract	0,5
Peptone Proteose Nº3	0,5	Casein hydrolysate	0,5
Magnesium Sulfate Anhydrous	0,024		

Preparation

Suspend 18,12 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until co mplete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 50 ºC, mix well and dispense into plates.

Instructions for use

» For clinical diagnosis, the type of sample is any sample of clinical origin.

- Surface inoculation with handle or swab.
- Incubate at 35±2 ºC for 5 days.
- Reading and interpretation of results.

» For other uses not covered by the CE marking.

According to Standard Methods for the Examination of Water and Wastewater:
- Prepare the dilutions for heterotrophic plate count.
- Inoculate the plates of R2 Agar by the pour plate method, spread method or the membrane filtration technique.
- Incubate at 20-28 ºC for 5-7 days and 35 ºC for 5-7 days.

According to European Pharmacopoeia for the microbial monitoring of water for Injections, water for preparations of extracts, and water purified:
- Filtrate the water with a membrane with a nomimal pore size not greater than 0,45 µm, using at least 200 ml of water for injections.
- Under normal conditions, an appropriate action level is a microbial count of 10 CFU per 100 ml when determinated by filtration.
- Inoculate plates of R2A Agar and incubate at 30-35 ºC for not less than 5 days.

For general Use:
- Inoculate plates with tap water samples using the streak plate technique and/or membrane filter method.
- Incubate at 35±2 ºC for 24-72 hours.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber	7,2 ± 0,2

Microbiological test

According to European Pharmacopoeia, Bacillus subtilis ATCC 6633 and Pseudomonas aeruginosa ATCC 9027:
Inoculation conditions: (<100 CFU)
Incubation conditions: (30-35 ºC / <3 days)

Rest of strains:
Incubation conditions: (35±2 ºC / 5 days)

Microorganisms	Specification
Staphylococcus epidermidis ATCC 12228	Good growth
Escherichia coli ATCC 25922	Good growth
Staphylococcus aureus ATCC 25923	Good growth
Staphylococcus aureus ATCC 6538	Good growth
Bacillus subtilis ATCC 6633	Good growth
Escherichia coli ATCC 8739	Good growth
Pseudomonas aeruginosa ATCC 9027	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

American Public Health Association (1985) Standard Method for the Enumeration of Water and Wasterwater.
European Pharmacopeia 9.0.