R2A Agar (NCM0076)

Revision: 3 Effective Date: 12/1/2021

Intended Use

R2A Agar is used for the enumeration and cultivation of bacteria from potable water in a laboratory setting and conforms to European Pharmacopoeia requirements. R2A Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

R2A Agar was developed by Reasoner and Geldreich for bacterial plate counts of treated potable water and is recommended in the European Pharmacopeia as a separate monograph. R2A Agar is a low nutrient medium, and in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria. Nutritionally rich media support the growth of fast-growing bacteria, and may suppress slow growing or stressed bacteria found in treated water. When compared with Tryptone Glucose Yeast Extract Agar or Plate Count Agar (Standard Methods Agar), R2A Agar reported improved recovery of stress and chlorine-tolerant bacteria from drinking water systems. R2A Agar is recommended in standard methods for pour plate, spread plate, and membrane filter methods for heterotrophic plate counts.

Typical Formulation

Enzymatic Digest of Casein	0.25 g/L
Enzymatic Digest of Animal Tissue	0.25 g/L
Acid Hydrolysate of Casein	0.5 g/L
Yeast Extract	0.5 g/L
Dextrose (Glucose)	0.5 g/L
Soluble Starch	0.5 g/L
Dipotassium Phosphate	0.3 g/L
Magnesium Sulfate Heptahydrate	0.05* g/L
Sodium Pyruvate	0.3 g/L
Agar	15.0 g/L

*Equivalent to 0.024 g/L Magnesium Sulfate Anhydrous

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 18.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.

Test Procedure

1. Prepare test dilutions for heterotrophic plate count.
2. Plate the test sample and dilutions by the spread plate, pour plate, or membrane filter method. Do not exceed 1 mL of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
3. Maintain proper humidity during prolonged incubation.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and light beige to beige.

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Prepared Appearance: Prepared medium is clear to slightly hazy and light beige.

Expected Cultural Response: Cultural response on R2A Agar incubated aerobically at 30-35°C and examined for growth at ≤72 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Enterococcus faecalis ATCC® 29212	10-100	70-200% recovery
Escherichia coli ATCC® 25922	10-100	70-200% recovery
Staphylococcus aureus ATCC® 25923	10-100	70-200% recovery
Aeromonas hydrophila ATCC® 7966	10-100	70-200% recovery
Bacillus subtilis ATCC® 6633	10-100	70-200% recovery
Pseudomonas aeruginosa ATCC® 9027	10-100	70-200% recovery

The organisms listed are the minimum that should be used for quality control testing.

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Results

Count colonies on spread or pour plates demonstrating 30 - 300 colonies per plate or 20 - 200 colonies when using the membrane filter method. Compute bacterial count per mL of sample by multiplying the average number of colonies per plate by the reciprocal of the appropriate dilution.
Report counts as colony forming units (CFU) per mL and report variables of incubation such as temperature and length of time.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the medium has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. R2A Agar is intended for use only with treated potable water.
3. Use of the pour plate method is discouraged because recovery of stressed bacteria may be compromised by the heat shock (44-46°C) and low oxygen tension that are part of the procedure.
4. Incubation time longer than indicated above may be necessary to recover additional slow-growing bacteria.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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