R2A Agar (Agar Medium S)

MAP962

(ME962/M962B)

Intended Use:

Recommended for heterotrophic plate count of treated potable water using longer incubation periods in accordance with EP/ BP.

Composition**

Ingredients	g / L
Casitose #	0.500
Yeast extract	0.500
Proteose peptone	0.500
Dextrose (Glucose)	0.500
Starch	0.500
Dipotassium hydrogen phosphate	0.300
Magnesium sulphate	0.024
Sodium pyruvate	0.300
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Casein hydrolysate

Directions

Suspend 18.12 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. DO NOT OVERHEAT. Cool to 45-50° C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Agar Medium S (R-2 A Agar) is used for the heterotrophic plate counts and for sub culturing isolates from potable waters using longer incubation periods was developed by Reasoner (1). The composition is as recommended by European Pharmacopoeia and British Pharmacopoeia (2,3). It is recommended for pour plate, spread plate and membrane filter techniques. Plate count recommended for the bacterial examination of potable waters, gives an estimate of the aerobic and facultatively anaerobic bacteria, which grow best at 35°C on a rich medium (4). However these organisms may represent a small number of total bacteria as other bacteria are either unable to grow under these conditions, or grow very slowly which cannot be detected in 48 hours.

R-2 A Agar is modified for better recovery of these bacteria from treated waters under different incubation conditions (4). Many bacteria from natural waters, which contain limited nutrients at ambient temperature, grow best on the media with less nutrient levels. Moreover, they grow better at the temperatures below the routine laboratory incubation temperatures of 35 to 37°C (4). R-2 A Agar, Modified is a low nutrient medium consisting of less proteose peptone, yeast extract and glucose as compared to Standard Methods Agar. This medium allows the growth of stressed, injured and chlorine tolerant bacteria present in treated waters due to the presence of pyruvate and starch. The number of colonies on a plate is reported as CFU (Colony Forming Units) per volume of sample.

Type of specimen

Pharmaceutical, water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standard (2,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Longer incubation time other than specified is required for slow growing microorganisms.
2. The media is intended for water samples for recovery of stressed or injured organisms.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

pH

7.2±0.2

Cultural Response

Cultural characteristics observed *by using standard ATCC cultures after an incubation at 30-35°C for 24-72 hours. (*-In case of water samples from fields it is suggested to incubate further for upto 7 days to ascertain the absence of organisms)

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	25 -100	>=50%
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	25 -100	>=50%
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	25 -100	>=50%
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50-100	good-luxuriant	25 -100	>=50%
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	good-luxuriant	25 -100	>=50%
** *Bacillus spizizenii* ATCC 6633 (00003*)	50-100	good-luxuriant	25 -100	>=50%
# *Aspergillus brasiliensis* ATCC 16404	50-100	good-luxuriant	25 -100	>=50%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	25 -100	>=50%
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant	25 -100	>=50%

Key : * Corresponding WDCM numbers.
**Formerly known as *Bacillus subtilis* subsp. *spizizenii*
^ Formerly known as *Pseudomonas aeruginosa*
# Formerly known as *Aspergillus niger*

Storage and Shelf Life

Store between 10-30°C in tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Reasoner and Geldreich, 1985, Appl. Environ. Microbiol., 49:1Collins and Willoughby, 1962, Arch. Microbiol., 43:294.
2. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
3. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
4. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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

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