

R-2A Agar

Intended Use:

Recommended for heterotrophic plate count of treated potable water using longer incubation periods.

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

R-2A Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts /slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

The heterotrophic plate count (HPC), formerly known as the standard plate count is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment, in distribution systems or in swimming pools. R-2A Agar is recommended by APHA (1,2) for estimating the heterotrophic plate count by the pour plate, spread plate or membrane filter procedure. R-2A Agar is formulated as per Reasoner and Geldreich (3). Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former (4). Therefore the use of a low nutrient medium like R-2A Agar incubated for longer incubation periods allows these stressed organisms to grow well as per EP and BP (5,6).

Many bacteria from natural waters which contain limited nutrients at ambient temperature, grow best on the media with less nutrient levels. They grow better at the temperatures below the routine laboratory incubation temperatures of 35 to 37°C (4).

Casitose, proteose peptone and yeast extract provide nitrogen, carbon compounds, vitamins, amino acids and minerals. Dextrose/ glucose serves as an energy source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Magnesium sulphate is a source of divalent cations and sulphate. Dipotassium hydrogen phosphate is used to balance the pH of the medium. The number of colonies on a plate are reported as CFU (Colony Forming Units) per volume of sample.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standard (1). 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. Further biochemical and serological testing is required for complete identification.

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

Sterile glass bottle containing slightly opalescent R-2A Agar.

Colour medium

Light amber coloured medium.

Quantity of medium

500 ml of medium in glass bottle.

pH

7.00-7.40

Sterility Check

Passes release criteria

Cultural Response

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 upto 7 days to ascertain the absence of organisms).

Organism	Inoculum (CFU)	Growth	Recovery
<i>Candida albicans</i> ATCC 10231 (00054*)	50-100	good-luxuriant	≥50%
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant	≥50%
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	≥50%
^ <i>Pseudomonas paraeruginosa</i> ATCC 9027 (00026*)	50-100	good-luxuriant	≥50%
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50-100	good-luxuriant	≥50%
** <i>Bacillus spizizenii</i> ATCC 6633 (00003*)	50-100	good-luxuriant	≥50%
# <i>Aspergillus brasiliensis</i> ATCC 16404(00053*)	50-100	good-luxuriant	≥50%
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50-100	good-luxuriant	≥50%
<i>Salmonella</i> Typhi ATCC 6539	50-100	good-luxuriant	≥50%

Key : (*) Corresponding WDCM numbers.

^ Formerly known as *Pseudomonas aeruginosa*

**Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Formerly known as *Aspergillus niger*

Storage and Shelf life

On receipt store between 15-30°C Use before expiry date on the label. 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 (7,8).

Reference

1. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
2. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
3. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.
4. Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.
5. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
6. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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. 1.66.

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