Technical Data

R-2A Broth (Double strength)

LQ571C

Intended use

Recommended for cultivation and maintenance of heterotrophic bacteria from potable waters.

Composition**

Ingredients	g/ L
Acicase#	1.000
Yeast extract	1.000
Proteose peptone	1.000
Dextrose (Glucose)	1.000
Starch soluble	1.000
Dipotassium hydrogen phosphate	0.600
Magnesium sulphate	0.048
Sodium pyruvate	0.600
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Casein Acid Hydrolysate

Directions

Label the ready to use R2A Broth, Double strength (LQ571C). Inoculate the sample and incubate at specified temperature and time.

Principle And Interpretation

The total bacterial count of drinking water is determined by plate count on a nutritionally rich medium. However all organisms present are not able to grow on them, either because they are slow growers or because they cant grow on that media (1). For this reason a nutritionally reduced medium was described. R-2A Agar is a modification of this medium (2). R-2A Agar is an alternative medium used for the heterotrophic plate counts and for subculturing isolates from potable waters (1). R-2A Agar is also recommended by APHA (3) for pour plate, spread plate and membrane filter technique. R-2A Broth is similar to R-2A Agar except agar. Total 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 in a rich medium . R-2A Broth enables better recovery of these bacteria from treated waters under different incubation conditions. 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). This medium contains acicase, yeast extract, proteose peptone as source of essential growth factors required for metabolism of the bacteria. Dextrose is the energy source. Starch acts as a neutralizer that neutralizes any toxic metabolites, if present. Phosphate buffers the medium while sodium pyruvate supplies additional nutrition. Magnesium sulphate serves as a source of ions. Due to the presence of the above mentioned ingredients these media allow the growth of stressed and chlorine tolerant bacteria present in treated waters.

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 (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.

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 clear R-2A broth, Double strength in glass bottles.

Colour

Light yellow coloured clear solution.

Quantity

100 ml of solution in glass bottles.

pH

7.00 - 7.40

Sterility Check

Passes release criteria

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
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50-100	good-luxuriant
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50-100	good-luxuriant
** *Bacillus spizizenii* ATCC 6633 (00003*)	50-100	good-luxuriant
# *Aspergillus brasiliensis* ATCC 16404	50-100	good-luxuriant
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant
Salmonella Typhi ATCC 6539	50-100	good-luxuriant

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

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 (4,5).

Reference

1.Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.
2.Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.
3.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
4.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5.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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