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TECHNICAL PRODUCT INFORMATION

R2A AGAR

Catalog No.	Description	Catalog No:	Description
P1525	20 mL	T1661	20 mL (pour tube)
P1526	25 mL	B1315	200 mL
P6030	10 mL (MF dish)	B4060	300 mL
P4012	Contact		

INTENDED USE:
This media is used for detection of heterotrophic bacteria in water, especially treated water. R2A Medium can be used as a pour plate, spread plate or membrane filter media[1] as a subculture medium.

HISTORY/SUMMARY:
R2A and R3A Media were developed by Reasoner and Geldreich of the U.S. Environmental Protection Agency (Cincinnati, OH) for the recovery and isolation of aerobic and facultative anaerobic heterotrophic bacteria from treated potable water samples. R2A Agar is a low nutrient medium, and in combination with a lower incubation temperature and longer incubation time, stimulates 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. The authors found R2A to be superior to Plate Count Agar when used in membrane filter, spread plate and pour plate methods.[2, 3]

PRINCIPLES OF THE PROCEDURE:
Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue and Acid Hydrolysate of Casein provide nitrogen, carbon and minerals. Yeast Extract is a source of vitamins and trace elements, Dextrose is a carbon source, and Soluble Starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Dipotassium Phosphate is used to balance the pH, Magnesium Sulfate Heptahydrate is a source of divalent cations and sulfate, Sodium Pyruvate increases the recovery of stressed cells and Agar is the solidifying agent.[4,5]

PRECAUTIONS:
Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established. This product is not for INVITRO DIAGNOSTIC USE.

STORAGE:
This medium should be stored at 2-8°C, use prior to expiration date.

PROCEDURE:
Pour Plate:
1. Pipet 1 mL of water sample into petri dish
2. Add 10-12 mL R2A at a temperature of 44-46°C.
3. Mix medium with sample.
4. Incubate at 35°C for at least 72 hours, but preferable 5-7 days.
5. If incubating at 20°-25°C; incubate for 5-7 days.

Spread Plate:
1. Pour 15 mL R2A into 100 mm dishes and allow solidifying.
2. Invert dishes and dry, use immediately.

3. Spread 1 mL of water sample evenly over agar.
4. Best recovery of results is from incubation at 22-25°C for 7 days[1].

Membrane Filter:

1. Dispense 5 mL into 50 mm dishes; allow solidifying.
2. Place filter membrane, through which the sample has been passed, onto the agar surface.
3. Take care not to trap any air between the filter and the agar surface.
4. Incubate at 35°C for a minimum of 48 hours.

Incubation Temperature	Minimum Incubation Time	Optimal Incubation Time
30-35°C	72 hours	5-7 days
20-25°C	5 days	7 days

RESULTS:

1. Spread or Pour Plates: count colonies on plates demonstrating 30-300 colonies per plate
2. Membrane Filtration: Count colonies on plates demonstrating 20-200 colonies per plate
3. Compute bacterial count per mL of samples by multiplying the average number of colonies per plate by the reciprocal of the appropriate dilution.
4. Report counts as colony forming units (CFU) per mL, report variables of incubation such as temperature and length of time.

FORMULA:

Ingredients per liter of demineralized water:
R2A Medium

Enzymatic Digest of Casein	0.25 g	Sodium Pyruvate	0.3 g
Enzymatic Digest of Animal Tissue	0.25 g	Soluble Starch	0.5 g
Acid Hydrolysate of Casein	0.5 g	Potassium Phosphate Dibasic	0.3 g
Yeast Extract	0.5 g	Magnesium Sulfate Heptahydrate	0.05 g
Dextrose	0.5 g	Agar	15.0 g

Final pH: 7.2 ± 0.2 at 25°C

TEST CHARACTERISTICS:

ORGANISMS	**RESULTS (when incubated at 35 ±0.2°C for 40-72 hours)**
ATCC# 25922 *Escherichia coli*	Growth
ATCC# 27853 *Pseudomonas aeruginosa*	Growth
ATCC# 29212 *Streptococcus faecalis*	Growth
ATCC# 25923 Staphylococcus aureus	Growth

LIMITATIONS OF PROCEDURE:

R2A is intended for use only with treated potable water, it is recommended for recovery of compromised bacteria.

Water sample testing[4, 5]:

All water samples should be tested as soon as possible without delay.

1. Samples not refrigerated, test within 6 hours of sample collection.
2. Refrigerated samples may be tested within 30 hours of sample collection

Use of the pour plate method is not recommended. Recovery of stressed bacteria may be compromised by the heat shock (44-46°C) and low oxygen tension produced by the procedure.
R2A performs best, with the spread plate technique; this procedure is limited to small sample volumes.

REFERENCES:

1. Standard Methods for Examination of Water and Wastewater 19th Edition, APHA. Washington DC, 1995
2. Reasoner, D.J. and E.E. Geldreich. Paper No. N7, Annual Meeting of the American Society for Microbiology, 1979
3. Reasoner, D.J. and E.E. Geldreich. Applied and Environmental Microbiology 49(1): 1-7, 1985
4. Difco & BBL Manual, Manual of Microbiological Culture Media, 2nd Edition, 2009, Page 461
5. Acumedia 7390 R2A Agar Product Information Sheet Rev 03 August 2009