Technical Information

Agar Medium S (R-2 A Agar)

Product Code: DM 1962B

Application: - Agar Medium S (R-2 A Agar) is recommended for heterotrophic plate count of treated potable water using longer incubation periods, in accordance with British Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Yeast extract	0.500
Casein hydrolysate	0.500
Proteose peptone	0.500
Starch	0.500
Glucose	0.500
Dipotassium hydrogen phosphate	0.300
Magnesium sulphate anhydrous	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

Principle & Interpretation

Agar Medium S (R-2 A Agar) is recommended for the heterotrophic plate counts and for sub culturing isolates from potable waters using longer incubation periods as per British Pharmacopoeia (1,2). It is used for pour plate, spread plate and membrane filter techniques. Plate count used 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 (3). 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 (3). 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 (3). 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 (2). The number of colonies on a plate is reported as CFU (Colony Forming Units) per volume of sample.

Methodology

Suspend 18.12 grams of dehydrated media in 1000 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. DO NOT OVERHEAT. Shake well before pour into sterile Petri plates.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

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

Reaction
After sterilization, reaction of 1.81% w/v aqueous solution. pH : 7.2±0.2

pH Range
7.00-7.40

Growth Promotion Test
As per British Pharmacopoeia.

Cultural Response
DM 1962B: Cultural characteristics observed *by using standard ATCC cultures after an incubation at 35-37°C for 24-72 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Candida albicans ATCC10231	50-100	good-luxuriant	>=50%
Enterococcus faecalis ATCC29212	50-100	good-luxuriant	>=50%
Salmonella Enteritidis ATCC13076	50-100	good-luxuriant	>=50%
Salmonella Typhi ATCC6539	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 8739	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. British pharmacopoeia, 2009, The Stationery office British Pharmacopoeia
2. Reasoner and Geldreich, 1985, Appl. Environ. Microbiol., 49:1.
3. Collins and Willoughby, 1962, Arch. Microbiol., 43:294.

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