Technical Information

R-2A Broth

Product Code: DM 2687

Application: - R-2A Broth is recommended for cultivation and maintenance of heterotrophic bacteria from potable waters.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate	0.500
Yeast extract	0.500
Proteose peptone	0.500
Dextrose	0.500
Starch, soluble	0.500
Dipotassium phosphate	0.300
Magnesium sulphate	0.024
Sodium pyruvate	0.300
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & 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 can grow on that media (1). For this reason a nutritionally reduced medium was described. R-2A Agar is a modification of this medium (2,3).

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 (4) 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 (3). 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 (3).

This medium contains casein acid hydrolysate, yeast extract, biopeptone 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.

Methodology

Suspend 3.12 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat if necessary to dissolve the medium completely. Dispense into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. DO NOT OVERHEAT.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity
Yellow coloured, clear solution in tubes

Reaction
Reaction of 0.312% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range
7.00-7.40

Cultural Response
DM 2687: 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 up to 7 days to ascertain the absence of organisms)

Organism	Inoculum (CFU)	Growth
Candida albicans ATCC 10231	50-100	good-luxuriant
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant
Escherichia coli ATCC 25922	50-100	good-luxuriant
Salmonella Enteritidis ATCC 13076	50-100	good-luxuriant
Salmonella Typhi ATCC 6539	50-100	good-luxuriant

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. Reasoner and Geldreich, 1985, Appl. Environ. Microbiol., 49:1. 2.Stark and McCoy. 1938. Zentralbl. Bacteriol. Parasitenkd. Infectionskr. Hyg. Abt.2 98 : 201
3. Collins and Willoughby, 1962, Arch. Microbiol., 43:294.
4.Greenberg A. E., Trussell R. R. and Clesceri L. S. (Eds.), 1985, Standard Methods for the Examination of Water and Wastewater, 16th ed., APHA, Washington, DC

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