R-3A Broth

M1688

R-3A Broth is recommended for sub culturing microorganisms from potable water.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate	1.000
Yeast extract	1.000
Biopeptone	1.000
Dextrose	1.000
Starch soluble	1.000
Dipotassium phosphate	0.600
Magnesium sulphate anhydrous	0.048
Sodium pyruvate	0.600
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 6.25 grams in 1000 ml distilled water. 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.

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 (2,3).

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).

R-3A Agar is a subculture medium which is used to subculture the organisms recovered on nutritionally deficient R-2A Agar (1). R-3A Broth is similar in composition to R-3A Agar, except agar.

These media contain 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.

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured clear solution in tubes

Reaction
Reaction of 0.625% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M1688: Cultural characteristics observed *by using standard ATCC cultures after an incubation at 35-37°C for 24-72 hours.

Organism	Inoculum (CFU)	Growth
Cultural Response		
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

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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.

Revision : 2 / 2015