Technical Data

R-2A HiCynth™ Agar

MCD962

R-2A HiCynth™ Agar is used for heterotrophic plate count of water samples using longer incubation periods.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.3*	0.500
HiCynth™ Peptone No.5*	1.000
Dextrose	0.500
Starch soluble	0.500
Dipotassium phosphate	0.300
Magnesium sulphate	0.024
Sodium puruvate	0.300
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptones

Directions

Suspend 18.12 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The heterotrophic plate count (HPC), formerly known as the standard plate count is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment, in distribution systems or in swimming pools. R-2A Agar is recommended by APHA (1, 2) for estimating the heterotrophic plate count by the pour plate, spread plate or membrane filter procedure. R-2A Agar is formulated as per Reasoner and Geldreich (3). Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former (4). Therefore the use of a low nutrient medium like R-2A Agar incubated for longer incubation periods allows these stressed organisms to grow well. R-2A HiCynth™ Agar is the modification of R-2A Agar by replacing animal peptones with chemically defined peptones to avoid BSE/TSE risk.

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).

HiCynth™ Peptone No.3 and HiCynth™ Peptone No.5 provide nitrogen, carbon compounds, long chain amino acids, vitamins and minerals. Dextrose serves as an energy source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Magnesium sulphate is a source of divalent cations and sulphate. Dipotassium phosphate is used to balance the pH of the medium. The number of colonies on a plate are reported as CFU (Colony Forming Units) per volume of sample.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 1.81% w/v aqueous solution at 25°C. pH : 7.20±0.20

pH

7.00-7.40

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)

Cultural Response

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

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. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
2. Downes F. P. and Ito K., (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
3. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.
4. Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.
5. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.

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