Heterotrophic Plate Count Agar

M1910

For heterotrophic plate count of bacteria in water

Composition**

Ingredients	Gms / Litre
Peptone	3.000
Soluble casein	0.500
Dipotassium hydrogen phosphate	0.200
Magnesium sulphate	0.050
Ferric chloride	0.001
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 18.75 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Heterotrophs are organisms including bacteria, yeasts and moulds that require an external source of organic carbon for growth. Heterotrophic Plate Count Method has been applied in many variants and is widely used to measure the heterotrophic microorganism population in drinking water systems (potable water), swimming pool and other waters (1,2). Three different methods are described for determining the heterotrophic plate count i.e. pour plate method, spread plate method and membrane filter method. The concentration of heterotrophic bacteria in the distribution system can be influenced by the bacteriological quality of the finished water entering the system, as well as water temperature, residence time, levels of disinfectant residual, pipe materials, surface area-to-volume ratio, flow conditions, and the availability of nutrients for growth(3).

Peptone and soluble Casein are the source of nutrients for organisms, which are not highly fastidious. Dipotassium hydrogen phosphate buffers the medium. Magnesium sulphate and ferric chloride are sources of inorganic ions.

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 1.88% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Bacillus subtilis ATCC 6633	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212	50-100	luxuriant	>=70%

Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=70%
Aeromonas hydrophila ATCC 7966	50-100	luxuriant	>=70%
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	>=70%
Citrobacter freundi ATCC 8090	50-100	luxuriant	>=70%
Acinetobacter calcoaceticus ATCC 23055	50-100	luxuriant	>=70%

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.Taylor R. H. and Geldreich E. E., 1979, J. Am. Water works Assoc. 71:402 .
2.Eaton A. D., Clesceri L. S. and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
3.Reasoner, 1990; Prévost et al., 1997; Payment, 1999; Carter et al., 2000; Clement et al., 2004

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