Heterotrophic Plate Count Agar

M1910

Intended Use:

Recommended for heterotrophic plate count of bacteria in water

Composition**

Ingredients	Gms / Litre
Peptone	3.000
M-Protein powder	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 purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. 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 (1,4). 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 (5).

Peptone and M-Protein powder provides nitogen, carbon compounds, long chain amino acids, vitamins and other 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.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological test must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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.

Organism	Inoculum (CFU)	Growth	Recovery
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	>=70%
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=70%
Aeromonas hydrophila ATCC 7966 (00063*)	50-100	luxuriant	>=70%
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%
Citrobacter freundi ATCC 8090	50-100	luxuriant	>=70%
Acinetobacter calcoaceticus ATCC 23055	50-100	luxuriant	>=70%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

3. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Taylor R. H. and Geldreich E. E., 1979, J. Am. Water works Assoc. 71:402 .

5. Reasoner, 1990; Prévost et al., 1997; Payment, 1999; Carter et al., 2000; Clement et al., 2004

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