

m-HPC Agar (NCM0284)

Intended Use

m-HPC Agar is used for the enumeration of heterotrophic organisms in water by the membrane filtration technique in a laboratory setting. m-HPC Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

m-HPC Agar, also known as m-Heterotrophic Plate Count Agar, was developed by Taylor and Geldreich in 1979. Monitoring the levels of heterotrophic bacteria, those microorganisms that can grow on minimal organic nutrients, is currently performed to indicate problems with treatment methods within distribution systems. There is evidence that heterotrophic bacteria are not hazardous to healthy individuals, but may be opportunistic pathogens capable of infecting individuals with impaired immune systems.

m-HPC Agar is used in standard methods for the detection of heterotrophic bacteria using the membrane filtration technique. The membrane filter technique is a microbiological method used in testing large volume samples. The concentration of larger samples on a membrane filter is a key benefit of the membrane filtration procedure. Several studies reported m-HPC Agar as a suitable alternate medium for standard total plate count agar.

Typical Formulation

Peptone 20.0 g/L
Gelatin 25.0 g/L
Agar 15.0 g/L
Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 60 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Add 10 mL of Glycerol to 1 Liter of solution.
4. Autoclave at 121°C for 5 minutes.
5. Cool to 45-50°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light beige.

Prepared Appearance: Prepared medium is trace to moderately hazy and colorless to light beige.

Expected Cultural Response: Cultural response on m-HPC Agar using the membrane filtration technique, incubated aerobically at 35°C and examined for growth after 24 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Enterococcus faecalis</i> ATCC® 29212	10 - 300	Growth
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Growth
<i>Pseudomonas aeruginosa</i> ATCC® 10145	10 - 300	Growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in standard methods or by laboratory policy.
2. Choose a sample size resulting in 20 - 200 colonies.
3. Transfer the filter to a prepared plate of m-HPC Agar, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes.
5. Invert plates and incubate at $35 \pm 0.5^{\circ}\text{C}$ for 24 - 48 hours.

Results

Count all the colonies on the membrane. Report average counts as estimated colony-forming units.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
2. m-HPC Agar is intended for use only with the membrane filtration procedure.
3. m-HPC Agar is recommended for testing treated water.
4. Longer incubation times may be necessary to recover slow-growing bacteria.

Storage

Store dehydrated culture media at $2-30^{\circ}\text{C}$ away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Taylor, R. H., and E. E. Geldreich. 1979. A new membrane filter procedure for bacteria counts in potable water and swimming pool samples. J. Amer. Water Works Assoc. 71:402-405.
2. <http://www.epa.gov/nerl/research/1999/html/g2-3.html>
3. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 2017. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C.
4. Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson. 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. J. Amer. Water Works Assoc. 73:585-590.
5. Nagy, L. A., and B. H. Olson. 1982. The occurrence of filamentous fungi in drinking water distribution systems. Can. J. Microbiol. 28:667-671.
6. Haas, C. N., M. A. Meyer, and M. S. Paller. 1982. Analytical note: evaluation of the m-SPC method as a substitute for the standard plate count in water microbiology. J. Amer. Water Works Assoc. 74:322.