m HPC Agar

Intended Use

m HPC Agar is used for enumerating heterotrophic organisms in treated potable water and other water samples with low counts by membrane filtration.

Summary and Explanation

m HPC Agar was developed by Taylor and Geldreich in 1979 in their pursuit of a suitable standard methods medium to use with the membrane filter procedure.[1] m HPC Agar is also known as m-Heterotrophic Plate Count Agar and previously as membrane filter Standard Plate Count Agar, m-SPC Agar. The formulation was evaluated by many investigators who reported it as a suitable alternative medium for standard plate counts.[2-4] It is recommended for the membrane filter method in the recent editions of *Standard Methods for the Examination of Water and Wastewater*.[5]

The advantages of the membrane filter procedure over the standard plate count method have been described by many investigators.[6-8] The volume of inoculum is limited with both pour and spread plate techniques while the membrane filter method enables the use of large samples, which is desirable for water with low counts.

Principles of the Procedure

Peptone provides nitrogen and carbon as well as other nutrients. The original concentration of 5% gelatin was reduced to 2.5% to avoid problems associated with liquefying gelatin and spreading colonies.

Formula

Difco™ m HPC Agar

Approximate Formula* Per Liter		
Peptone	20.0	g
Gelatin	25.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 6 g of the powder in 100 mL of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 1 mL of glycerol.
4. Autoclave at 121°C for 5 minutes. Cool to 45-50°C.
5. Dispense 5 mL portions into 50 × 9 mm Petri dishes.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Excessive heat may cause breakdown of the gelatin. See "Limitations of the Procedure."

Procedure

Water samples should be collected and handled as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060.[5]

1. The volume to be filtered will vary with the sample. Select a maximum sample size to give 20-200 CFU per filter.
2. Filter the appropriate volume through a sterile 47 mm, 0.45 µm, gridded membrane filter, under partial vacuum. Rinse funnel with three 20-30 mL portions of sterile dilution water. Place filter on agar in Petri dish.

User Quality Control

Identity Specifications

Difco™ m HPC Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6% solution, soluble in purified water upon boiling. (Add 1% glycerol after boiling.) With glycerol, solution is light amber, slightly opalescent to opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, opalescent, may have a precipitate.
Reaction of 6% Solution with Glycerol at 25°C:	pH 7.1 ± 0.2

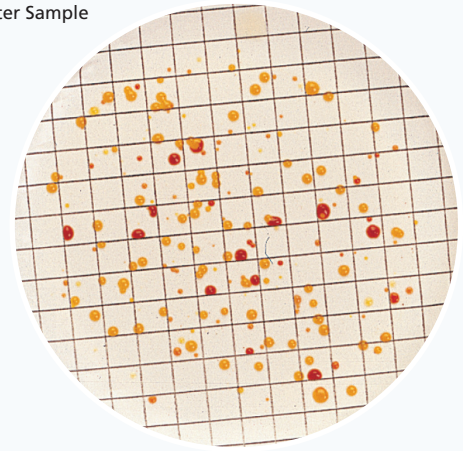
Cultural Response

Difco™ m HPC Agar

Prepare the medium per label directions. Inoculate using the membrane filtration technique. Incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212	20-200	Growth
Escherichia coli	25922	20-200	Growth
Pseudomonas aeruginosa	10145	20-200	Growth

Water Sample



3. Place dishes in a close-fitting box or plastic bag containing moistened paper towels.
4. Incubate at 35 ± 0.5°C for 48 hours. Duplicate plates may be incubated at other conditions as desired.

Expected Results

Count all colonies on the membrane when there are 2 or less colonies per square. For 3-10 colonies per square, count 10 squares and obtain average count per square. For 10-20 colonies per square, count 5 squares and obtain average count per square. Multiply average count per square by 100 and divide by the sample volume to give colonies per milliliter. If there are more than 20 colonies per square, record count as > 2,000 divided by the sample volume. Report averaged counts as estimated colony-forming units. Make estimated counts only when there are discrete, separated colonies.[5]

Limitations of the Procedure

1. m HPC Agar is intended for use only with the membrane filter method.
2. m HPC Agar is recommended for testing treated water.
3. Longer incubation times may be necessary to recover slow-growing bacteria.
4. This medium may not be sterile; use with care to avoid contamination.

References

1. Taylor and Geldreich. 1979. J. Am. Water Works Assoc. *71*:402.
2. Means, Hanami, Ridgway and Olson. 1981. J. Am. Water Works Assoc. *73*:585.
3. Nagy and Olson. 1982. Can. J. Microbiol. *28*:667.
4. Haas, Meyer and Paller. 1982. J. Am. Water Works Assoc. *74*:322.
5. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
6. Lechevallier, Seidler and Evans. 1980. Appl. Environ. Microbiol. *40*:922.
7. Stapert, Sokolski and Northam. 1962. Can. J. Microbiol. *8*:809.
8. Saleem and Schlitzer. 1983. Abstr. Q 126, p. 281. Abstr. 83rd Annu. Meet. Am. Soc. Microbiol. 1983.

Availability

Difco™ m HPC Agar

COMPF SMWW

Cat. No.	275220	Dehydrated – 500 g

Difco™ Glycerol

Cat. No.	228210	Bottle – 100 g
	228220	Bottle – 500 g