IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

M-HETEROTROPHIC PLATE COUNT (HPC) AGAR

Cat. no. G95	m-HPC Agar, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. G195	m-HPC Agar, 15x100mm Plate, 20ml	10 plates/bag

INTENDED USE

Hardy Diagnostics m-HPC Agar is recommended for the enumeration of heterotrophic microorganisms in water samples by the membrane filtration technique.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

The heterotrophic plate count method is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment and distribution or in the analysis of samples from recreational waters such as swimming pools.

Hardy Diagnostics m-HPC Agar is a high nutrient medium designed for use in measuring heterotrophic bacteria in water by the membrane filtration technique. The membrane filtration technique allows the testing of large volumes of low-turbidity water and is the method of choice for low-count waters 1 to 10 CFU/ml), as recommended by the American Public Health Association (APHA).[1,5,6]

m-HPC Agar consists of gelatin and peptone, which provide a source of nutrients for non-fastidious microorganisms. Glycerol is added to the medium to provide a source of carbon and energy. Agar is the solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

Gelatin	25.0gm
Peptone	20.0gm
Glycerol	10.0ml
Agar	15.0gm

Final pH 7.1 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store the product at 2-8ºC. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample Collection: Consult listed references for information on sample collection.[1-7]

Method of Use[1]

1. Filter appropriate volume of water sample through a sterile 47mm, 0.45µm, gridded membrane filter, under partial vacuum for Cat. no. G95. Cat. no. G195 can be used similarly and can accommodate a membrane filter of up to 82mm in diameter.

Note: Select a maximum sample size to give 20 to 200 CFU per filter.

2. Rinse funnel with three 20 to 30ml portions of sterile dilution water.

3. Place the filter on the surface of the m-HPC Agar taking care to avoid entrapment of air bubbles between the agar and filter surfaces.

4. Place the inoculated plates in a close fitting box or plastic bag containing moistened paper towels.

5. Incubate the plates aerobically at 35ºC. for 48 hours.

Note: Duplicate plates may be incubated under varying time and temperature conditions as desired.

INTERPRETATION OF RESULTS

Colonies on membrane filters are counted using a stereoscopicmicroscope at a magnification of 20 to 15X.

The petri dish should be slanted at a 45 degree angle on themicroscope stage. The light source should be adjusted vertical to the colonies. The optimal colony density per filter is 20 to 200.

All colonies on the membrane filter are counted when there are ≤ 2 colonies per square. When 3 to 10 colonies per square are present,ten squares are counted and an average count per square is calculated. When 10 to 20 colonies per square are present, five squares are countedand an average counter per square is calculated.

The average number of colonies per milliliter is obtained by multiplying the average count per square by 100 and divide by the sample volume.

When 20 or more colonies per square are present, record thecount as > 2000 divided by the sample volume.

Average counts are reported as estimated colony forming units. Estimated counts are made only when colonies are separated and there are no spreaders.

Refer to listed references for further information concerning computing and reporting counts.[(1)]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, stereoscopic microscopes, membrane filters, incinerator, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus aureus ATCC® 25923	MF	up to 48hrs	35°C	Aerobic	Growth / 10-100 colonies
Enterococus faecalis ATCC® 29212	MF	up to 48hrs	35°C	Aerobic	Growth / 10-100 colonies
Pseudomonas aeruginosa ATCC® 27853	MF	up to 48hrs	35°C	Aerobic	Growth / 10-100 colonies
Escherichia coli ATCC® 25922	MF	up to 48hrs	35°C	Aerobic	Growth / 10-100 colonies

* Refer to the document "Inoculation Procedures for Media QC" for more information.

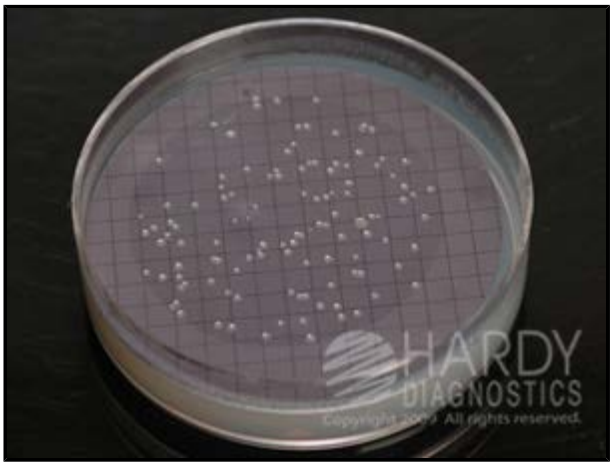
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable

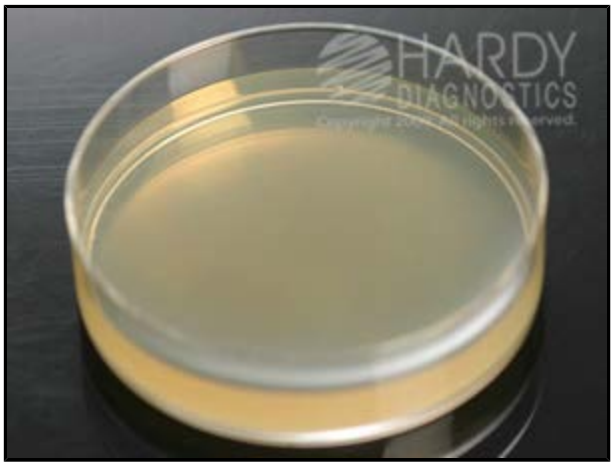
government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

m-HPC Agar should appear clear, slightly opalescent and light amber in color.



Escherichia coli (ATCC® 25922) filtered through a black membrane (Cat. no. A045R047A) and growing on m-HPC Agar (Cat. no. G95). Incubated aerobically for 24 hours at 35ºC.



Uninoculated plate of m-HPC Agar (Cat. no. G95).

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater* , APHA, Washington, D.C.

2. *Association of Official Agricultural Chemists* , 10th ed. 1965. p. 737.

3. Association of Official Analytical Chemists. *Official Methods of Analysis* , AOAC, Washington, D.C.

4. The Official Compendia of Standards. 2008. *USP27-NF22* . United States Pharmacopeial Convention, Rockville, MD.

5. American Public Health Association. *Standard Methods for the Examination of Dairy Products* , APHA, Washington, D.C.

6. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

7. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]