IFU

Instructions for Use

HAEMOPHILUS TEST MEDIUM (HTM) AGAR

Cat. no. G33	HTM Agar, 15x100mm Plate, 25ml	10 plates/bag
Cat. no. H07	HTM Agar, 15x150mm Plate, 72ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Haemophilus Test Medium (HTM) Agar is a solid medium recommended by the Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS) for use in antimicrobial disk susceptibility tests of *Haemophilus* species.[4]

SUMMARY

Increasing incidence of resistant strains of *Haemophilus influenzae* has underscored the need for a reliable susceptibility test method for this organism. Modifications of standard procedures have become necessary because of the fastidious nature of *H. influenzae* . Complex growth additives may cause antagonism of certain antibiotics and have shown poor reproducibility. Jorgensen, et al. described this formula, Haemophilus Test Medium, as an improved growth medium for *Haemophilus influenzae*.[5] In their study, the HTM compared favorably with the conventional medium, Mueller Hinton with Chocolate Agar. HTM is an uncomplicated growth medium that is transparent to facilitate the reading of susceptibility tests.

FORMULA

Ingredients per liter of deionized water:*

Acid Hydrolysate of Casein	17.5gm
Yeast Extract	5.0gm
Beef Extract	2.0gm
Starch	1.5gm
Hematin	15.0mg
NAD	15.0mg
Agar	17.0gm

Final pH 7.3 +/- 0.1 at 25ºC.

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

STORAGE AND SHELF LIFE

Upon receipt store 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration, (shrinking, cracking or discoloration), or if the expiration date has passed.

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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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

Specimen Collection: This product is not intended for primary isolation of patient specimens. This medium is used in characterizing pure cultures. Direct inoculation of this medium will result in erroneous results. Information on specimen collection may be found in standard reference texts.[1,3]

Method of Use: Refer to CLSI document M2-A.[4]

INTERPRETATION OF RESULTS

Refer to CLSI document M2-A.[4]

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.

Failure to follow CLSI recommended procedures as described in the standard document may result in inaccurate results.[4]

Bacterial suspensions must be prepared according to CLSI recommended procedure and compared with the appropriate turbidity standard to insure reliable results. Higher inoculum concentrations may lead to false resistant results with some beta-lactamase antimicrobics.[4,5]

The plates are to be incubated at 35ºC., in 5-7% CO_2, for 16-18 hours. A longer incubation time may lead to erroneous results; i.e. zones that are too small.

Disk diffusion methods for HTM have been standardized only for *Haemophilus* spp. Other organisms should not be

tested because results cannot be reliably interpreted.

The use of other strains of *H. influenzae* for quality control testing other than the strains listed may lead to erroneous results.

A nitrocefin beta-lactamase test should be performed on all *Haemophilus* isolates. beta-lactamase negative, ampicillin-resistant strains of *Haemophilus influenzae* are rare in the United States (< 0.5%).

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, incinerators, incubators, and antimicrobial disks, 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	Incubation			Results
	Time	Temperature	Atmosphere	
Haemophilus influenzae ATCC® 49247	16-18hr	35°C	CO_2**	Refer to CLSI document M2 for QC and interpretive zone diameters
Haemophilus influenzae ATCC® 49766	16-18hr	35°C	CO_2**	Refer to CLSI document M2 for QC and interpretive zone diameters
Haemophilus influenzae ATCC® 10211	16-18hr	35°C	CO_2**	Tested for growth only
Escherichia coli ATCC® 35218	16-18hr	35°C	Aerobic	Refer to CLSI document M2 for QC and interpretive zone diameters

* 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

HTM Agar should appear clear, and medium to dark amber in color.

Haemophilus influenzae (ATCC® 49247) growing on Haemophilus Test Medium (Cat. no. H07) for antimicrobial disk susceptibility test. Incubated in CO_2 for 18 hours at 35ºC.

Uninoculated plate of Haemophilus Test Medium (Cat. no. H07) for antimicrobial disk susceptibility test. Incubated in CO_2 for 18 hours at 35ºC.

PERFORMANCE CHARACTERISTICS

A reproducibility study was performed involving three separate test sites. Ten *Haemophilus influenzae* isolates, five ATCC® strains and five clinical isolates, were tested in triplicate at each of the three test sites on three separate days (10x3x3x3).

Clinical disk diffusion testing of HTM at an independent test site resulted in < 4% no growth rate of *Haemophilus* spp.

Reproducibility results are available from Hardy Diagnostics upon request. Acceptable criteria ≥ 95% reproducibility.

See listed reference for more information on reproducibility of clarithromycin on HTM.[6]

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

2. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. *Performance Standards for Antimicrobial Disk Susceptibility Tests*, M2-A. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

5. Jorgensen, J.H., et al. 1987. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. *J. Clin. Microbiol.*; 25:2105-2113.

6. Jones, R.N., et al. 1994. Validation of NCCLS macrolides; azithromycin, clarithromycin and erythromycin interpretive criteria for *Haemophilus influenzae* tested with Haemophilus Test Medium. *Diagn. Microbiol. Infect. Dis.*; 18: 243-249.

7. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*, Informational Supplement. Approved Standard M100. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

ATCC is a registered trademark of the American Type Culture Collection.

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

HDQA 2207F [D]