IFU

Instructions for Use

MUELLER HINTON WITH CHOCOLATE

Cat. no. E20	Mueller Hinton with Chocolate, 15x100mm Plate, 24ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Mueller Hinton with Chocolate is an enriched medium recommended for the isolation and cultivation of fastidious microorganisms, particularly *Haemophilus* species.

SUMMARY

In the 1960s, Bauer, Kirby, et al. developed Mueller Hinton medium for use in the disk diffusion procedure for determining susceptibility testing of bacteria to antibiotic and chemotherapeutic agents.[2] The medium was also developed for the cultivation of pathogenic *Neisseria* spp.[7] Since fastidious microorganisms grew poorly, the medium was eventually supplemented with 1% hemoglobin and adopted for testing of *Haemophilus influenzae*. Consequently, Mueller Hinton with Chocolate was formerly recommended by the Clinical Laboratory Standards Institute (CLSI) for susceptibility testing of *H. influenzae*.[6,9] More recent methods of disk diffusion testing have replaced Mueller Hinton with Chocolate with Haemophilus Test Medium (HTM), Cat. no. G33 or H07. However, Mueller Hinton with Chocolate is still recommended by CLSI for antimicrobial susceptibility testing of bacteria isolated from animals.[10]

Mueller Hinton with Chocolate contains beef extract and casein which provide nitrogenous components, amino acids, vitamins and minerals to support microbial growth. Starch is added to neutralize toxic fatty acids present in the agar. Hemoglobin provides hemin (X-factor) for the growth of *Haemophilus* spp. The medium is also supplemented with KoEnzyme enrichment, a chemically defined supplement that provides NAD (V-factor), amino acids, vitamins, dextrose, ferric ions, and coenzymes to promote the growth of pathogenic *Neisseria* species.

FORMULA

Ingredients per liter of deionized water:*

Acid Hydrolysate of Casein	17.5gm
Hemoglobin	10.0gm
Beef Extract	2.0gm
Starch	1.5gm
KoEnzyme Enrichments	10.0ml
Agar	17.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. 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 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: Consult listed references for information on specimen collection.[2-5,8,10] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Method of Use:

1. Allow plates to acclimate to room temperature prior to use.

2. Streak the specimen to obtain isolated colonies. Alternatively, if the specimen is obtained from a swab, roll the swab over a small portion of the agar surface and streak to obtain isolated colonies.

3. Incubate plates at 35ºC. for 18-24 hours, and up to 72 hours, in an aerobic atmosphere enriched with 5-10% CO_2.

INTERPRETATION OF RESULTS

Observe plates for the growth of isolated colonies within the streak zone. The growth of *Haemophilus* spp. may appear as small (1.0mm), moist, pearly colonies with a characteristic "mousy" odor.

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, swabs, applicator sticks, other culture media, incinerators, 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	
Haemophilus influenzae ATCC® 10211	A	24hr	35°C	CO_2**	Growth

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

** Atmosphere of incubation is enriched with 5-10% CO_2.

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

Mueller Hinton with Chocolate should appear opaque, smooth and brown in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Bauer, A.W., W.M.M. Kirby, et al. 1966. *Am. J. Clin. Pathol.*; 45:493-496.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. *Methods for Dilution Antimicrobial Susceptibility Test For Bacteria That Grow Aerobically*, M7-current edition.

Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.

7. Mueller, J.H. and J. Hinton. 1941. A protein-free medium for primary isolation of the *Gonococcus* and *Meningococcus*. *Proc. Soc. Exp. Diol. and Med.*; 48:330-333.

8. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

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

10. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*; *Approved Standard*. M31-A. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

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

HDQA 2207F [D]