IFU

Instructions for Use

CRITERION™ MUELLER HINTON AGAR

Cat. no. C6420	CRITERION™ Mueller Hinton Agar	76gm
Cat. no. C6421	CRITERION™ Mueller Hinton Agar	500gm
Cat. no. C6422	CRITERION™ Mueller Hinton Agar	2kg
Cat. no. C6423	CRITERION™ Mueller Hinton Agar	10kg
Cat. no. C6424	CRITERION™ Mueller Hinton Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Mueller Hinton Agar is recommended for use in the cultivation of a wide variety of microorganisms. Mueller Hinton Agar is recommended for disk diffusion sensitivity testing of non-fastidious organisms.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

Mueller and Hinton developed Mueller Hinton Agar in 1941 to be a protein-free medium for isolating pathogenic strains of *Neisseria*.[10] In recent times this media has been used in standardized antimicrobial disk susceptibility testing, as described by Bauer, Kirby, et al.[5] Barry and Fay investigated the effects of altering the depth of plated Mueller Hinton Agar on disk diffusion testing, and determined a standardized depth of approximately four millimeters to be sufficient.[4] In 1970 Dewees, et al., studied the effect of storage on Mueller Hinton Agar plates used for antimicrobial disk diffusion zone sizes. Their findings indicated commercially manufactured Mueller Hinton Agar plates were suitable for use in routine susceptibility testing.[6] In addition to the above criteria, CRITERION™ Mueller Hinton Agar meets the standards of performance established by the Clinical Laboratory Standards Institute (CLSI), documents M2, M100, and ISO 16782.[11,15,16]

CRITERION™ Mueller Hinton Media contains beef infusion and casamino acids, and starch. Starch acts as a colloid that protects against toxic material in the medium. Beef infusion and casamino acids are provided as a source of energy and nutrients. Agar is added as a solidifying agent. The levels of tetracycline and sulfonamide inhibitors, thymidine, thymine, magnesium and calcium ions are controlled so as not to interfere with susceptibility testing and to yield good growth.

The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. Disk diffusion testing is based on an antimicrobial diffusing through an agar gel, when placed on the agar surface after it has been impregnated onto a filter paper disk.[11,14] Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganisms are listed in the Clinical Laboratory Standards Institute (CLSI), document M100.[15]

FORMULA

Gram weight per liter:	38.0gm/L
Acid Hydrolysate of Casein	17.5gm
Beef Extract	2.0gm
Starch	1.5gm
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

Store the sealed bottle(s) containing culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original beige.

Store the prepared plated media at 2-8ºC.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 38.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes. Avoid overheating.

4. Cool to 50-55ºC. and dispense to give an approximate depth of 4mm. Refer to CLSI (NCCLS) document M2-A.[11]

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. H11.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

The disk diffusion method should not be used for obligatory anaerobes, slow growing organisms, and capnophiles. This method was standardized for facultative organisms or rapid growing aerobes.

When using the disk diffusion method, technical human errors may compromise reliability and accuracy. The following errors are common sources encountered in the clinical microbiology laboratory, and must be watched for: improper disk storage, inoculum not properly adjusted (too light or too heavy), incubation temperature deviating from 35-37ºC., use of an increased CO_2 atmosphere, reading plates before or after the full 16-18 hours of incubation, transcribing errors, reader error when measuring zone diameters, deterioration of the McFarland Turbidity Standard, and contamination or mutation in the control strain(s).

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., 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	
Escherichia coli ATCC® 25922	F	24hr	35°C	Aerobic	Growth**
Escherichia coli ATCC® 35218	F	24hr	35°C	Aerobic	Growth**
Staphylococcus aureus ATCC® 25923	F	24hr	35°C	Aerobic	Growth**
Pseudomonas aeruginosa ATCC® 27853	F	24hr	35°C	Aerobic	Growth**
Enterococcus faecalis					

ATCC® 29212	F	24hr	35°C	Aerobic	Growth**
Enterococcus faecalis ATCC® 33186	F	24hr	35°C	Aerobic	Growth**
Staphylococcus aureus ATCC® NCTC 12493	F	24hr	35°C	Aerobic	Growth**
Staphylococcus aureus ATCC® 43300	F	24hr	35°C	Aerobic	Growth**

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

**For appropriate disk diffusion ranges, consult CLSI M100 and ISO 16782.[15,16]

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Mueller Hinton Agar powder should appear homogeneous, free-flowing and beige, with a few dark specks, in color. The prepared media should appear translucent, and light amber 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. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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

4. Barry and Fay. 1973. *Am. J. Clin. Pathol.*; 50:196.

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

6. Dewees, et al. 1970. Effect of storage of Mueller Hinton Agar plates on zone sizes for antimicrobial testing. *Appl. Microbiol.*; 30:203.

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

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

9. *Methods for Dilution Antimicrobial Susceptibility Test For Bacteria That Grow Aerobically*, M7-current edition. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.

10. 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.

11. *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 7th ed. M2-A7. 2000. Clinical Laboratory

Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

13. Ryan, K.J., et al. 1970. Disk sensitivity testing. *Hosp. Prac.*; 5:91-100.

14. Standard Disk Susceptibility Test, The Federal Register, September 30, 1972; 37(191):20527-20529.

15. *Performance Standards for Antimicrobial Susceptibility Testing*. M100. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.

16. ISO 16782. Clinical laboratory testing - Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing.

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 2207B [D]