

# Instructions for Use

## MUELLER HINTON MEDIA

<a href="#">Cat. no. G45</a>	Mueller Hinton Agar, 15x100mm Plate, 28ml	10 plates/bag
<a href="#">Cat. no. H11</a>	Mueller Hinton Agar, 15x150mm Plate, 69ml	10 plates/bag
<a href="#">Cat. no. K38</a>	Mueller Hinton Broth, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. K91</a>	Mueller Hinton Broth, 16x100mm Tube, 5ml	20 tubes/box
<a href="#">Cat. no. R38</a>	Mueller Hinton Broth, 13x100mm Tube, 3ml	20 tubes/box

## INTENDED USE

Hardy Diagnostics Mueller Hinton Media 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. Mueller Hinton Broth is recommended for preparing suspensions of microorganisms for disk diffusion sensitivity testing.

## SUMMARY

Mueller and Hinton developed Mueller Hinton Agar in 1941 to be a protein free medium for isolating pathogenic strains of *Neisseria*.<sup>(6)</sup> It was found that Mueller Hinton Agar was useful in identifying sulfonimide-resistant and responsive strains of gonococci.<sup>(9)</sup> Additionally, this media has been used in standardized antimicrobial disk susceptibility testing, as described by Bauer, Kirby, et al.<sup>(2)</sup> 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.<sup>(1)</sup> 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.<sup>(3)</sup> In addition to the above criteria, Hardy Diagnostics Mueller Hinton Agar meets the standards of performance established by the Clinical and Laboratory Standards Institute (CLSI), M2, M100, and ISO 16782 documents.<sup>(7,13)</sup>

Mueller Hinton Media contains beef infusion, casamino acids, and starch. Starch acts as a colloid that protects against toxic material in the medium. Beef infusion and casamino acids provide energy and nutrients. Agar is added when a solidifying agent is needed. 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.<sup>(11)</sup>

The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. It is based on the use of an antimicrobial impregnated filter paper disk. The impregnated disk is placed on an agar surface, resulting in diffusion of the antimicrobial into the surrounding medium. Effectiveness of the antimicrobial can be shown by measuring the zone of inhibition for a pure culture of an organism.<sup>(7,10)</sup> Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganisms are listed in the Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing*, and M100.<sup>(12)</sup>

Mueller Hinton Broth is the same formulation, without the added agar. It is used for the cultivation of microorganisms, and for making dilutions of organisms to be used in the Kirby-Bauer disk diffusion procedure.

## FORMULA

Ingredients per liter of deionized water:\*

Acid Hydrolysate of Casein	17.5gm
Beef Extract	2.0gm
Starch	1.5gm

In addition, Mueller Hinton Agar contains:

Agar	17.0gm
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Final pH 7.3 +/- 0.1 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store plates at 2-8°C. away from direct light. Tubed media may be stored at 2-30°C. 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.<sup>(4)</sup> 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: For Mueller Hinton Agar, refer to CLSI documents M2-A, M7-A, and M100.<sup>(5,7,12)</sup>

Method of Use: For Mueller Hinton Broth, inoculate as per recognized practices for the cultivation of organisms.<sup>(5,7)</sup>

## INTERPRETATION OF RESULTS

Consult CLSI document M100.<sup>(12)</sup>

## 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.

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.

*In vitro* susceptibility does not necessarily imply *in vivo* effectiveness.

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 35-37°C, use of an increased CO<sub>2</sub> 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).

Research shows that strains of enterococci lacking thymidylate synthetase are folate dependent and may fail to grow on thymidine-thymine deficient media.<sup>(11)</sup> In addition, media containing exogenous end products of the folate pathway may affect the results of organisms tested with trimethoprim-sulfamethoxazol, as sulfonamide and trimethoprim activity may be inhibited or reduced on media containing elevated levels of thymidine and para-aminobenzoic acid (PABA) or its analogs.<sup>(11)</sup>

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, antimicrobial disks, McFarland 0.5 Turbidity Standard, forceps, 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	
<b>Mueller Hinton Broth:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	24hr	35°C	Aerobic	Growth

<i>Staphylococcus aureus</i> ATCC® 25923	A	24hr	35°C	Aerobic	Growth
<i>Enterococcus faecalis</i> ATCC® 29212	A	24hr	35°C	Aerobic	Growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	A	24hr	35°C	Aerobic	Growth
<i>Escherichia coli</i> ATCC® 35218	A	24hr	35°C	Aerobic	Growth
<b>Mueller Hinton Agar:</b>					
<i>Escherichia coli</i> ATCC® 25922	F	24hr	35°C	Aerobic	Growth**
<i>Escherichia coli</i> ATCC® 35218	F	24hr	35°C	Aerobic	Growth**
<i>Staphylococcus aureus</i> ATCC® 25923	F	24hr	35°C	Aerobic	Growth**
<i>Pseudomonas aeruginosa</i> ATCC® 27853	F	24hr	35°C	Aerobic	Growth**
<i>Enterococcus faecalis</i> ATCC® 29212	F	24hr	35°C	Aerobic	Growth**
<i>Enterococcus faecalis</i> ATCC® 33186	F	24hr	35°C	Aerobic	Growth**
<i>Staphylococcus aureus</i> ATCC® NCTC 12493	F	24hr	35°C	Aerobic	Growth**
<i>Staphylococcus aureus</i> 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.<sup>(12,13)</sup>

## 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 Agar should appear translucent, and light amber in color.
- Mueller Hinton Broth should appear clear, and very light amber in color; may have slight precipitate.



*Escherichia coli* (ATCC® 25922) growing on Mueller Hinton Agar (Cat. no. H11) for antimicrobial disk susceptibility test. Incubated aerobically for 24 hours at 35°C.



*Enterococcus faecalis* (ATCC® 29212) growing on Mueller Hinton Agar (Cat. no. H11) for antimicrobial disk susceptibility test. Incubated aerobically for 24 hours at 35°C.



*Pseudomonas aeruginosa* (ATCC® 27853) growing on Mueller Hinton Agar (Cat. no. H11) for antimicrobial disk susceptibility test. Incubated aerobically for 24 hours at 35°C.



Uninoculated plate of Mueller Hinton Agar (Cat. no. H11).

## REFERENCES

1. Barry and Fay. 1973. *Am. J. Clin. Pathol.*; 50:196.
2. Bauer, A.W., W.M.M. Kirby, et al. 1966. *Am. J. Clin. Pathol.*; 45:493-496.
3. Dewees, et al. 1970. Effect of storage of Mueller Hinton Agar plates on zone sizes for antimicrobial testing. *Appl. Microbiol.*; 30:203.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. *Methods for Dilution Antimicrobial Susceptibility Test For Bacteria That Grow Aerobically*, M7-current edition. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.
6. Mueller, J.H. and J. Hinton. 1941. A protein-free medium for primary isolation of the *Gonococcus* and *Meningococcus* *Proc. Soc. Exp. Biol. and Med.*; 48:330-333.

7. *Performance Standards for Antimicrobial Disk Susceptibility Tests* . M2-A. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
9. Ryan, K.J., et al. 1970. Disk sensitivity testing. *Hosp. Prac.*; 5:91-100.
10. Standard Disk Susceptibility Test. *The Federal Register*, September 30, 1972; 37(191):20527-20529.
11. Haltiner, R.C., P.C. Migneault, and R.G. Roberston. 1980. Incidence of Thymidine-Dependent Enterococci Detected on Mueller-Hinton Agar with Low Thymidine Content. *Anti. Ag. and Chemo.*; 18(3): 365-368
12. *Performance Standards for Antimicrobial Susceptibility Testing*. M100. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.
13. 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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