

MUELLER HINTON AGAR (7101)

Intended Use

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to National Committee for Clinical Laboratory Standards (NCCLS).¹

Product Summary and Explanation

Mueller Hinton Agar is based on the formula recommended by Mueller and Hinton² for the primary isolation of *Neisseria species*. Mueller and Hinton selected pea meal extract agar as a simple transparent medium containing heat stable ingredients.³ During their modification, starch replaced the growth-promoting properties of pea extract, acting as a “protective colloid” against toxic substances.

Bauer, Kirby, Sherris and Tuck⁴ recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This unsupplemented medium has been selected by the National Committee for Clinical Laboratory Standards (NCCLS)¹ for several reasons:⁵ this medium is low in sulfonamide, trimethoprim and tetracycline inhibitors, provides satisfactory growth of most non-fastidious pathogens and demonstrates batch-to-batch reproducibility.

Mueller Hinton Agar is often abbreviated as M-H Agar, and complies with requirements of the World Health Organization.⁵ Mueller Hinton Agar is specified in FDA Bacteriological Analytical Manual⁶ for food testing, and procedures commonly performed on aerobic and facultatively anaerobic bacteria.⁷ A variety of supplements can be added to Mueller Hinton Agar, including 5% defibrinated sheep or horse blood, 1% growth supplement and 2% sodium chloride.

Principles of the Procedure

Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent.

A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula / Liter

Beef Extract	2 g
Acid Hydrolysate of Casein	17.5 g
Starch.....	1.5 g
Agar	17 g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 38 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. OPTIONAL: Supplement as appropriate. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature.
5. Check prepared Mueller Hinton Agar to ensure the final pH is 7.3 ± 0.1 at 25°C.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is slightly opalescent with no significant precipitation, and light to medium amber.

Expected Cultural Response: Prepare, inoculate and dispense antibiotic disks following the procedure described by NCCLS.^{1,8,9} The cultures listed should have middle range zone sizes of the concentration tested.⁸

Microorganism	Response & Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	growth; zone diameters within published specifications
<i>Escherichia coli</i> ATCC® 25922	growth; zone diameters within published specifications
<i>Escherichia coli</i> ATCC® 35218	growth; zone diameters within published specifications
<i>Pseudomonas aeruginosa</i> ATCC® 27853	growth; zone diameters within published specifications
<i>Staphylococcus aureus</i> ATCC® 25923	growth; zone diameters within published specifications
<i>Staphylococcus aureus</i> ATCC® 43300	growth; zone diameters within published specifications

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on antimicrobial susceptibility testing, refer to procedures outlined in appropriate references.

Results

Refer to appropriate documents for correct zone sizes.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH. Strict adherence to protocol is required to ensure reliable results.⁹
3. Drug inactivation may result from the prolonged incubation times required by slow growers.¹⁰
4. Variation in the concentration of divalent cations, primarily calcium and magnesium affects result of aminoglycoside, tetracycline, and colistin test with *P. aeruginosa* isolates.⁷

Packaging

Mueller Hinton Agar	Code No.	7101A	500 g
		7101B	2 kg
		7101C	10 kg

References

1. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA.
2. **Mueller, J. H., and J. Hinton.** 1941. A protein-free medium for primary isolation of gonococcus and meningococcus. Proc. Soc. Exp. Biol. Med. **48**:3330-333.
3. **Gordon and Hine.** 1916. Br. Med. J. **678**.
4. **Bauer, A. L., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493-496.
5. **World Health Organization.** 1961. Standardization of methods for conducting microbic sensitivity tests. Technical Report Series No. 210, Geneva.
6. **Food and Drug Administration.** Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
7. **Wood, G. L., and J. A. Washington.** 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods, p. 1327-1341. In Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **National Committee for Clinical Laboratory Standards.** 1996. Protocols for evaluating dehydrated; App. Standard. Wayne PA.
9. **National Committee for Clinical Laboratory Standards.** 1999. M100-S9. Performance Standards for Antimicrobial Susceptibility Testing; Ninth Informational Supplement. Wayne, PA.
10. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.