MUELLER HINTON AGAR (7101)

Intended Use

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to Clinical and Laboratory Standard Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS).[1]

Product Summary and Explanation

Mueller Hinton Agar is based on the formula recommended by Mueller and Hinton[2] 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 Tuck[4] recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This unsupplemented medium has been selected by the Clinical and Laboratory Standard Institute (CLSI)[1] for several reasons. This medium is low in sulfonamide, trimethoprim and tetracycline inhibitors, and provides satisfactory growth of most non-fastidious pathogens along with demonstrating 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 Manual[6] 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 hazy and light to medium yellow.

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

Microorganism	Response & Reactions
Enterococcus faecalis ATCC® 29212	Growth; zone diameters within published specifications
Escherichia coli ATCC® 25922	Growth; zone diameters within published specifications
Escherichia coli ATCC® 35218	Growth; zone diameters within published specifications
Pseudomonas aeruginosa ATCC® 27853	Growth; zone diameters within published specifications
Staphylococcus aureus ATCC® 25923	Growth; zone diameters within published specifications
Staphylococcus aureus 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 antimicrobic 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. 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]
2. Drug inactivation may result from the prolonged incubation times required by slow growers.[10]
3. 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. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A9. CLSI, 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. **www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.**
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. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **Clinical and Laboratory Standards Institute.** 2006. Protocols for evaluating dehydrated Mueller Hinton Agar, 2nd ed.; Approved standard M6-A2, CLIS, Wayne PA.
9. **Clinical and Laboratory Standards Institute.** 2008. Standards for Antimicrobial Susceptibility Testing; Eighteenth informational supplement, M100-S18 (MS). 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 (517)372-9200 or fax us at (517)372-2006.

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