PRODUCT SPECIFICATION SHEET

Mueller Hinton Agar (DM172)

Intended Use
Mueller Hinton Agar (DM172) is used for cultivation of Neisseria and for determination of susceptibility of microorganisms to antimicrobial agents.

Product Summary and Explanation
Mueller Hinton Agar was originally developed for the cultivation of pathogenic *Neisseria.* [6] However, these organisms are now commonly isolated on selective media. In the early 1960s clinical microbiology laboratories used a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents. Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium.[1,2] A subsequent international collaborative study confirmed the value of Mueller Hinton Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium.[7] The CLSI has written a performance standard for the Bauer-Kirby procedure and this document should be consulted for additional details.[4] The procedure is recommended for testing rapidly growing aerobic or facultatively anaerobic bacterial pathogens, such as staphylococci, members of the *Enterobacteriaceae*, aerobic gram-negative rods; e.g., *Pseudomonas* spp. and *Acinetobacter* spp., enterococci and *Vibrio cholerae*. The procedure is modified for testing fastidious species; i.e., *H. influenzae*, *N. gonorrhoeae* and *S. pneumoniae* and other streptococci.
Mueller Hinton Agar is manufactured to contain low levels of thymine and thymidine[8,9] and controlled levels of calcium and magnesium.[10-12] Thymine and thymidine levels of raw materials are determined using the disc diffusion procedure with trimethoprim-sulfamethoxazole (COT) discs and *Enterococcus faecalis ATCC 33186* and/or *29212*. Calcium and magnesium levels are controlled by testing raw materials and supplementing with sources of calcium and/or magnesium as required to produce correct zone diameters with aminoglycoside antibiotics and *Pseudomonas aeruginosa* ATCC 27853.[13]
Mueller Hinton agar complies with requirements of the World Health Organization[14] and is specified in the FDA *Bacteriological Analytical Manual* for food testing.15 Unsupplemented Mueller Hinton agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms such as *S. pneumoniae*. The CLSI Document M2*, Performance Standards for Antimicrobial Disk Susceptibility Tests*, recommends Mueller Hinton agar supplemented with 5% defibrinated sheep blood. Details of quality control procedures and interpretive criteria for use with *S. pneumoniae* and other *Streptococcus* spp. Are contained in supplemental tables.[5] These documents should be consulted for additional details.[4,5]

Principles of the Procedure
Acid hydrolysate of casein and beef extract supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support the growth of microorganisms. Starch is added to absorb any toxic metabolites produced and acts as a protective colloid against toxic substances. Hydrolysis of the starch during autoclaving provides a small amount of dextrose, which is a source of energy. 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.

PRODUCT SPECIFICATION SHEET

Formula / Liter

Ingredients	Gms / Litre
Beef, infusion	300.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 38 grams of the medium in 1000 ml of distilled water/purified water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 lbs pressure, for 15 minutes.
4. Mix well before pouring.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow, homogeneous, free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in the petri plates
Reaction of 3.8% Solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.7% Agar gel

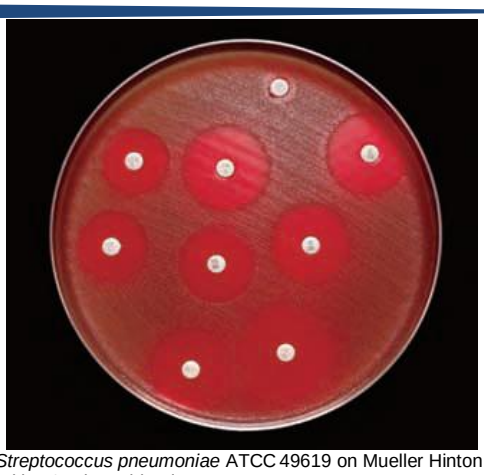
Expected Cultural Response: Cultural response on Mueller Hinton Agar at 35 - 37°C for 18 - 24 hours. of incubation.

Sr No.	Organisms	Inoculum (CFU)	Growth	Recovery
1.	*Escherichia coli ATCC 25922*	50-100	Luxuriant	>=70%
2.	*Haemophilus influenzae ATCC 49247*	50-100	Good-luxuriant (on Mueller Hinton Chocolate Agar)	>=70%
3.	*Neisseria gonorrhoeae ATCC 49226*	50-100	Luxuriant	>=70%
4.	*Enterococcus faecalis ATCC 29212*	50-100	Luxuriant	>=70%
5.	*Streptococcus pneumonia ATCC 6305*	50-100	Luxuriant (on Mueller Hinton Blood Agar)	>=70%
6.	*Escherichia coli ATCC 35218*	50-100	Luxuriant	>=70%
7.	*Staphylococcus aureus ATCC 43300*	50-100	Luxuriant	>=70%
8.	*Pseudomonas Aeruginosa ATCC 77853*	50-100	Luxuriant	>=70%
9.	*Staphylococcus aureus ATCC 25923*	50-100	Luxuriant	>=70%

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

PRODUCT SPECIFICATION SHEET

Pseudomonas Aeruginosa ATCC 27853 on Mueller Hinton Agar



Streptococcus pneumoniae ATCC 49619 on Mueller Hinton Agar with 5% sheep blood

Test Procedure

For a complete discussion on antimicrobic susceptibility testing, refer to procedures outlined in appropriate references.[19,20] Protocols developed by the CLSI and used by manufacturers to evaluate the performance of Mueller Hinton Agar in comparison to a reference medium are published in CLSI document M6-A.[21]

Results

Zone diameters measured around discs should be compared with those in the CLSI Document M100 (M2). Results obtained with specific organisms may then be reported as resistant, intermediate or susceptible.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if 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.[22]
2. When Mueller Hinton agar is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2-3 mm smaller than those obtained with unsupplemented agar.[23] Conversely, sheep blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.[24] Sheep blood may cause indistinct zones or a film of growth within the zones of inhibition around sulfonamide and trimethoprim discs.[23]
3. Mueller Hinton agar deeper than 4 mm may cause false resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.[23]
4. A pH outside the range of 7.3 ± 0.1 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity.[23] The opposite effects are possible if the pH is too high.[23]

PRODUCT SPECIFICATION SHEET

Packaging
Product Name: Mueller Hinton Agar (DM172)
Product Code : DM172
Available Pack sizes : 100gm / 500gm

References

1. Bauer, Kirby, Sherris and Turck. 1966. Am. J. Clin. Pathol. *45*:493.
2. Ryan, Schoenknecht and Kirby. 1970. Hospital Practice *5*:91.
3. Barry, Garcia and Thrupp. 1970. Am. J. Clin. Pathol. *53*:149.
4. Clinical and Laboratory Standards Institute. 2006. Approved standard: M2-A9. Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.
5. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement, M100-S18(M2). CLSI, Wayne, Pa.
6. Mueller and Hinton. 1941. Proc. Soc. Exp. Biol. Med. *48*:330.
7. Ericsson and Sherris. 1971. Acta Pathol. Microbiol. Scand. Sec. B, Suppl. 217.
8. Koch and Burchall. 1971. Appl. Microbiol. *22*:812.
9. Ferone, Bushby, Burchall, Moore and Smith. 1975. Antimicrob. Agents Chemother. *7*:91.
10. Reller, Schoenknecht, Kenny and Sherris. 1974. J. Infect. Dis. *130*:454.
11. Pollock, Minshew, Kenny and Schoenknecht. 1978. Antimicrob. Agents Chemother. *14*:360.
12. D'Amato and Thornsberry. 1979. Current Microbiol. *2*:135.
13. Thornsberry, Gavan and Gerlach. 1977. Cumitech 6, New developments in antimicrobial agent susceptibility testing. Coord. ed., Sherris. American Society for Microbiology, Washington, DC.
14. World Health Organization. 1961. Standardization of methods for conducting microbic sensitivity tests. Technical Report Series No. 210, Geneva, Switzerland.
15. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
16. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, DC.
17. Hindler and Anderbied. 1985. J. Clin. Microbiol. *21*:205.
18. Baker, Thornsberry and Hawkinson. 1983. J. Clin. Microbiol. *17*:450.
19. Koneman, Allen, Janda, Schreckenberger and Winn. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott Raven Publishers, Philadelphia, Pa.
20. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
21. Clinical and Laboratory Standards Institute. 2006. Approved standard: M6-A2. Protocols for evaluating dehydrated Mueller-Hinton agar, 2nd ed. CLSI, Wayne, Pa.
22. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
23. Wood and Washington. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
24. Buschelman, Jones and Bale. 1994. J. Clin. Microbiol. *32*:565.

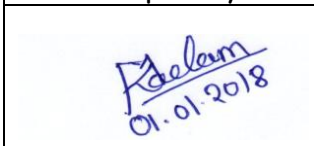
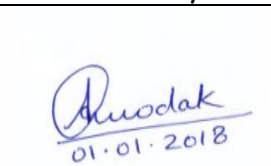
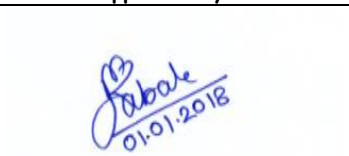
Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

DM172PSS,QAD/FR/024,Rev.00/01.01.2018

PRODUCT SPECIFICATION SHEET

Prepared By	Checked By	Approved By
		
Microbiologist	Head Quality Control	Head Quality Assurance

Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

PRODUCT SPECIFICATION SHEET