MUELLER HINTON AGAR

INTENDED USE: s used in antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to EUCAST (European Committee on Antimicrobial Susceptibility Testing).

SUMMARY AND EXPLANATION OF THE TEST: Mueller Hinton Agar is based on the formula recommended by Mueller and Hinton for the primary isolation of *Neisseria* species. Mueller and Hinton selected pea meal extract agar as a simple transparent medium containing heat stable ingredients. 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 recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This unsupplemented 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. Mueller Hinton Agar is specified in FDA Bacteriological Analytical Manual for food testing, and procedures commonly performed on aerobic and facultatively anaerobic bacteria. 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 ..... 2,0 g
Acid Hydrolysate of Casein..... 7,5 g
Starch..... 1,5 g
Agar ..... 17,0 g

SUPPLEMENTS:

FINAL pH: 7,3 ± 0,1 at 25°C.

DIRECTIONS FOR PREPARATION FROM DEHYDRATED PRODUCT: Suspend 38,0 g of the medium in one liter of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes.

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

EXPECTED RESULTS: Refer to appropriate documents for correct zone sizes.

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 EUCAST. The cultures listed should have middle range zone sizes of the concentration tested.[8]

Microorganism	Expected Results
Escherichia coli ATCC 25922	Growth
Staphylococcus aureus ATCC 29213	Growth
Pseudomonas aeruginosa ATCC 27853	Growth
Enterococcus faecalis ATCC 29212	Growth

STORAGE: ready to use plates – 6-12°C;

PACKAGING: **cat No. 201051** ready to use plates Ø 90 mm (1 x 10 pcs);

EXPIRATION: ready to use plates – 90 days;

REFERENCES:

1. 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.
2. Gordon and Hine. 1916. Br. Med. J. 678.
3. 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 PI 7101 Rev 3, June 2011.
4. World Health Organization. 1961. Standardization of methods for conducting microbic sensitivity tests. Technical Report Series No. 210, Geneva.
5. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.
6. 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.
7. Clinical and Laboratory Standards Institute. 2006. Protocols for evaluating dehydrated Mueller Hinton Agar, 2nded.; Approved standard M6-A2, CLIS, Wayne PA.
8. Clinical and Laboratory Standards Institute. 2008. Standards for Antimicrobial Susceptibility Testing; Eighteenth informational supplement, M100-S18 (MS). Wayne, PA.
9. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.
10. http://www.eucast.org/antimicrobial_susceptibility_testing/

Staphylococcus aureus ATCC 29213

Antimicrobial agent	Disc content (µg)	Inhibition zone diameter (mm)	
		Target[1]	Range[2]
Amikacin	30	21	18-24
Benzylpenicillin	1 unit	15	12-18
Cefoxitin	30	27	24-30
Chloramphenicol	30	24	20-28
Ciprofloxacin	5	24	21-27
Clindamycin	2	26	23-29
Erythromycin	15	26	23-29
Fusidic acid	10	29	26-32
Gentamicin	10	22	19-25
Levofloxacin	5	-	-
Linezolid	10	24	21-27
Minocycline	30	-	-
Moxifloxacin	5	28	25-31
Netilmicin	10	23	20-26
Nitrofurantoin	100	20	17-23
Norfloxacin	10	21	18-24
Ofloxacin	5	24	21-27
Quinupristin-dalfopristin	15	-	-
Rifampicin	5	33	30-36
Tetracycline	30	27	23-31
Tigecycline[4]	15	22	19-25
Tobramycin	10	23	20-26
Trimethoprim	5	25	22-28
Trimethoprim-sulfamethoxazole	1.25-23.75	29	26-32

[1] Calculated by Quality Control Department (Graso)

[2] Established and validated by EUCAST.

Pseudomonas aeruginosa ATCC 27853

Antimicrobial agent	Disc content (µg)	Inhibition zone diameter (mm)	
		Target[1]	Range[2]
Amikacin	30	22	18-26
Aztreonam	30	26	23-29
Cefepime	30	27	24-30
Ceftazidime	10	24	21-27
Ciprofloxacin	5	29	25-33
Doripenem	10	32	28-35
Gentamicin	10	19	16-21
Imipenem	10	24	20-28
Levofloxacin	5	23	19-26
Meropenem	10	30	27-33
Netilmicin	10	18	15-21
Piperacillin-tazobactam	30-6	26	23-29
Ticarcillin-clavulanic acid	75-10	24	20-28
Tobramycin	10	22	19-25

[1] Calculated by Quality Control Department (Graso)

[2] Established and validated by EUCAST.

Escherichia coli ATCC 25922

Antimicrobial agent	Disc content (µg)	Inhibition zone diameter (mm)	
		Target[1]	Range[2]
Amikacin	30	23	19-26
Amoxicillin	10	-	-
Amoxicillin-clavulanic acid	20-10	21	18-24
Ampicillin	10	19	16-224
Ampicillin-sulbactam	10-10	22	19-24
Aztreonam	30	32	28-36
Cefadroxil	30	17	14-20
Cefalexin	30	-	-
Cefepime	30	34	31-37
Cefixime	5	25	23-27
Cefotaxime	5	28	25-31
Cefpodoxime	10	26	23-28
Ceftazidime	10	26	23-29
Ceftibuten	30	31	27-35
Ceftriaxone	30	32	29-35
Cefuroxime	30	23	20-26
Chloramphenicol	30	24	21-27
Ciprofloxacin	5	35	30-40
Doripenem	10	31	27-35
Ertapenem	10	33	29-36
Gentamicin	10	23	19-26
Imipenem	10	29	26-32
Levofloxacin	5	33	29-37
Mecillinam	10	27	24-30
Meropenem	10	31	28-34
Moxifloxacin	5	32	28-35
Nalidixic acid	30	25	22-28
Netilmicin	10	-	-
Nitrofurantoin	100	20	17-23
Norfloxacin	10	32	28-35
Ofloxacin	5	31	29-33
Piperacillin	30	24	21-27
Piperacillin-tazobactam	30-6	24	21-27
Ticarcillin	75	27	24-30
Ticarcillin-clavulanic acid	75-10	27	24-30
Tigecycline5	15	24	20-27
Tobramycin	10	22	18-26
Trimethoprim	5	25	21-28
Trimethoprim-sulfamethoxazole	1.25-23.75	26	23-29

[1] Calculated by Quality Control Department (Graso)

[2] Established and validated by EUCAST.

Enterococcus faecalis ATCC 29212

Antimicrobial agent	Disc content (µg)	Inhibition zone diameter (mm)	
		Target[1]	Range[2]
Ampicillin	2	18	15-21
Gentamicin	304	15	12-18
Imipenem	10	27	24-30
Linezolid	10	22	19-25
Nitrofurantoin	100	21	18-24
Quinupristin-dalfopristin	15	14	11-17
Teicoplanin	30	18	15-21
Tetracycline	30	13	10-16
Tigecycline5	15	23	20-26
Trimethoprim	5	28	24-32
Trimethoprim-sulfamethoxazole	1.25-23.75	30	26-34
Vancomycin	5	13	10-16

[1] Calculated by Quality Control Department (Graso)

[2] Established and validated by EUCAST.

Graso Biotech
Krąg 4A, 83-200 Starogard Gdański
Customer service: 58 562 30 21, fax. 58 562 79 87,
zamowienia@graso.com.pl; www.podloza.pl, www.grasobiotech.pl