Mueller Hinton Agar

M173

Intended Use:

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Composition**

Ingredients	Gms / Litre
HM infusion B from #	300.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.4±0.1

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef infusion from

- Equivalent to Casein acid hydrolysate

Directions

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (6). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.

ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.

iii. It supports the growth of most non-fastidious bacterial pathogens and

iv. Many data and much experience regarding its performance have been recorded (7).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (11). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for

Enterococcus faecalis with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (2,6,9). A standardized suspension of the organism is swabbed over the entire surface

$ The zones for these discs are indicative of the Divalent Cation content of the medium

Cultural Response

Organism	Growth	Standard Zone	Zone of inhibition Observed
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		
Cephalothin CEP 30mcg		29-37 mm	29 -37 mm
Chloramphenicol C 30 mcg		21-27 mm	21 -27 mm
Co-Trimoxazole COT 25 mcg #		23-29 mm	23 -29 mm
Cefotaxime CTX 30 mcg		29-35 mm	29 -35 mm
Gentamicin GEN 10 mcg		19-26 mm	19 -26 mm
Sulphafurazole SF 300 mcg		15-23 mm	15 -23 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		
Co-Trimoxazole COT 25 mcg #		# 20 mm (Clear zone)	>=20 mm
Cefoxitin CX 30 mcg		23-29 mm	23 -29 mm
Erythromycin E 15 mcg		22-30 mm	22 -30 mm
Linezolid LZ 30 mcg		25-32 mm	25 -32 mm
Oxacillin OX 1mcg		18-24 mm	18 -24 mm
Pristinomycin RP 15 mcg		21-28 mm	21 -28 mm
Tetracycline TE 30 mcg $		18-25 mm	18 -25 mm
Ciprofloxacin CIP 5mcg		22-30 mm	22 -30 mm
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		
Ceftazidime CAZ 30 mcg		22-29 mm	22 -29 mm
Ciprofloxacin CIP 5mcg		30-40 mm	30 -40 mm
Tobramycin TOB 10 mcg $		19-25 mm	19 -25 mm
Amikacin AK 30 mcg $		18-26 mm	18 -26 mm
Aztreonam AT 3mcg		23-29 mm	23 -29 mm
Cephotaxime CTX 30 mcg		18-22 mm	18 -22 mm
Gentamicin GEN 10 mcg $		16-21 mm	16 -21 mm
Imipenem IPM 10 mcg		20-28 mm	20 -28 mm
Piperacillin PI 100 mcg		12-18 mm	25 -33 mm
***Escherichia coli* ATCC 35218**	luxuriant		
Amoxyclav AMC 30 mcg		18-24 mm	18 -24 mm
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm	24 -30 mm
Ticarcillin TI 75 mcg		6 mm	6 -6 mm
Ticarcillin/Clavulanic acid TCC 75/10mcg		20-28 mm	20 -28 mm
Ampicillin AMP 10 mcg		16-22 mm	16 -22 mm
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm	29 -37 mm
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		
Trimethoprim TR 5 mcg #		# 20 mm	>=20 mm
Vancomycin VA 30 mcg		17-21 mm	17 -21 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 43300 (MRSA) (00211*)**	luxuriant		
Oxacillin OX 1 mcg		Very Hazy to No Zone	No zone

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

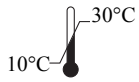
Reference

1. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
2. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C. ,,
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
8. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa.
9. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.
10. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards.
11. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.

Revision : 04 / 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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