Mueller Hinton Agar

SM173

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)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef infusion from

- Equivalent to Casein acid hydrolysate

Directions

Mueller Hinton Agar is a ready to use solid media in glass bottle.The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts /slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

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 of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (7). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (7,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (5).

Type of specimen

Clinical samples : Isolated microorganism from urine , stool, blood etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Sterile Mueller Hinton Agar in glass bottle.
Colour of medium
Light amber coloured medium
Quantity of medium
100 ml of medium in glass bottle.
Reaction
7.20-7.40
Sterility test
Passes release criteria

Cultural Response
Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures. For testing S.pneumoniae : The medium was supplemented with 5% Sheep blood and incubated at 35°C for 16-18 hours at 5% CO2 For testing *H.influenaze* : The medium was supplemented with 5g/l of Yeast extract & 2 vials /l of Haemophilus Growth Supplement (FD117 containing 15 mg/l of Haematin + 15 mg/l of NAD) and incubated at 35°C for 20-24 hours at 5% CO2
Antibiotic Sensitivity test
Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100)
Thymine/Thymidine Content
The zones for these discs are indicative of the Thymine/Thymidine content of the medium.
Divalent Cation Content

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

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

On receipt store between 15-25°C 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.

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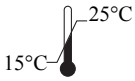
In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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