microgen
Ready Prepared Media

Technical Information

Mueller Hinton Agar Plate

Product Code: PM 1173

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

Composition**

Ingredients	Gms / Litre
HM infusion solids B # (from 300g)	2.000
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 heart infusion
- Equivalent to Casein acid hydrolysate

Principle & Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (1). 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 (2). 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 (4).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (5). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (6). 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 imethoprim (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 (7,1,2). 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 (4). 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 (4,8). 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 (9).

Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through anagar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical andLaboratory Standards Institute) Approved Standard (10).

Type of specimen

Clinical samples : Pure cultures isolated from urine , stool, blood etc.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,10-13). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use only. For professional 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.

Methodology

Either streak, inoculate or surface spread the test inoculum aseptically on the plate.

Quality Control

Appearance
Sterile Mueller Hinton Agar 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles
Colour of medium
Light amber coloured medium
Quantity of medium
25 ml of medium in 90 mm disposable plates.
pH
7.20-7.40
Sterility Check
Passes release criteria
Cultural Response
Antibiotic susceptibility tests are performed in accordance with, and meet the acceptance limits of the current ISO/TS 16782 (15). Performance of the medium is checked in accordance with the CLSI/ EUCAST guidelines.
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	Incubation Temperature	Incubation Period
Escherichia coli ATCC	luxuriant		34-36°C	16-20 hours

25922 (00013*)				
Cephalothin CEP 30mcg		15-21mm		
Ampicillin AMP 10mcg		15-22mm		
Chloramphenicol C 30 mcg		21-27mm		
Gentamicin GEN 10mcg		19-26mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		23-29mm		
Sulphafurazole SF 300 mcg		15-23mm		
Cefotaxime CTX 5 mcg		25-31mm		
Tigecycline TGC 15mcg		20-27mm		
Tetracycline TE 30 mcg		18-25mm		
Amoxicillin- clavulanate AMC 30 mcg		18-24mm		
Ciprofloxacin CIP 5mcg		29-38mm		
Escherichia coli ATCC 35218	luxuriant		34-36°C	16-20 hours
Amoxicillin- clavulanate AMC 30 mcg		17-22 mm		
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm		
Ticarcillin TI 75 mcg		6 mm		
Ticarcillin/Clavulanic acid TCC 75/10mcg		21-25mm		
Ampicillin AMP 10 mcg		6 mm		
Ampicillin/Sulbactam A/S 10/10 mcg		13-19 mm		
Staphylococcus aureus subsp. aureus ATCC	luxuriant		34-36°C	16-20 hours
25923 (00034*)				
Erythromycin E 15 mcg		22-30mm		
Linezolid LZ 30 mcg		24-30mm		
Tetracycline TE 30 mcg		24-30mm		
Ciprofloxacin CIP 5mcg		22-30mm		
Amoxyclav(Amoxicillin/ Clavulanic acid) AMC 30 mcg		28-36mm		
Co-Trimoxazole COT 25 mcg		24-32mm		
Cefoxitin CX 30 mcg		23-29mm		
Oxacillin OX 1mcg		18-24mm		
Pristinomycin RP 15 mcg		21-28mm		
Gentamicin GEN 10 mcg		19-27mm		
Penicillin-G 10 units		26-37mm		
Ampicillin/Sulbactam A/S 10/10 mcg		29-37mm		
Staphylococcus aureus subsp. aureus ATCC	luxuriant		34-36°C	16-20 hours
29213 (00131*)				
Penicillin-G P 1 unit		12-18 mm		
Cefoxitin CX 30 mcg		24-30 mm		
Erythromycin E 15 mcg		23-29 mm		
Linezolid LZ 10 mcg		21-27 mm		
Gentamicin GEN 10 mcg		19-25 mm		
Tetracycline TE 30 mcg $		23-31 mm		
Ciprofloxacin CIP 5mcg		21-27 mm		
Staphylococcus aureus subsp. aureusATCC	luxuriant		34-36°C	24 hours

43300 (MRSA) (00211*)				
Oxacillin OX 1 mcg		Very Hazy to No Zone		
Cefoxitin CX 30 mcg		<=21 mm		
Pseudomonas aeruginosa ATCC 27853 (00025*)	luxuriant		34-36°C	24 hours
Ceftazidime CAZ 30 mcg		22-29 mm		
Ciprofloxacin CIP 5mcg		25-33 mm		
Tobramycin TOB 10 mcg $		20-26 mm		
Amikacin AK 30 mcg $		20-26 mm		
Aztreonam AT 3mcg		23-29 mm		
Cephotaxime CTX 30 mcg		18-22 mm		
Gentamicin GEN 10 mcg $		17-23 mm		
Imipenem IPM 10 mcg		20-28 mm		
Piperacillin PI 100 mcg		25-33mm		
Piperacillin Tazobactum PIT 30/6 mcg		23-29 mm		
Enterococcus faecalis ATCC 29212 (00087*)	luxuriant		34-36°C	24 hours
Trimethoprim TR 5 mcg #		24-32 mm		
Ampicillin AMP 2 mcg		15-21mm		
Imipenem IPM 10 mcg		24-30mm		
Linezolid LZ 10 mcg		19-25mm		
Nitrofurantoin NIT 100 mcg		18-24mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		26-34mm		
Vancomycin VA 5 mcg		10-16mm		
Enterococcus faecalis ATCC33186 (00210*)	luxuriant		34-36°C	16-20 hours
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		<=20 mm		

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 20-30°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 (3,5).

Further Reading

1. 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.
2. 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.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
5. 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.
6. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
7. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.
8.NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd

Ed.,National Committee for Clin. Lab. Standards.
9.Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
10.Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.
11.ISO/TS 16782:2016, Confirmed in 2021 Clinical laboratory testing - Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing
12.European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 14.0, valid from 2024-01-01.
13.European Committee on Antimicrobial Susceptibility Testing Routine and extended internal quality control as recommended by EUCAST Version 14.0, valid from 2024-01-01.

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- User must ensure suitability of the product(s) in their application prior to use.
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