Mueller Hinton Agar No. 2 Plate

MP1084

Intended Use:

Recommended for testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by the Kirby-Bauer method.

Composition**

Ingredients	g / L
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

Directions

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

Principle And Interpretation

The goal of susceptibility test is to predict through an in vitro assessment the likelihood of successfully treating a patient's infection with a particular antimicrobial agent (1). The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (2). 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 (3). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard (4). 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 (1). Mueller Hinton Agar No. 2 is used in the susceptibility testing of rapidly growing aerobic and facultatively anaerobic bacteria from clinical specimens. 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). The medium is designed to give a low thymine and thymidine content and also the calcium and magnesium ion concentration is adjusted as recommended by CLSI (3). The medium is not recommended for fastidious organisms. Thymine and thymidine inhibit sulfonamide and trimethoprim (7,8) activity and calcium and magnesium (9,10) interferes with the activity of aminoglycoside antibiotics. HM infusion solids B 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). Calcium and magnesium ion concentrations are adjusted to provide the amounts recommended by CLSI to give the correct MIC values with aminoglycosides and *Pseudomonas aeruginosa* (3) 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 (2,3,11). 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 (12), ISO (15) and EUCAST standards (16-17). 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 (1,12).

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 (13,14).
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.

Quality Control

Appearance

Sterile Mueller Hinton Agar No. 2 Plate in 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 25922 (00013*)**	luxuriant		34-36°C	16-20 hours
Cephalothin CEP 30mcg		15-21 mm		
Ampicillin AMP 10mcg		15-22 mm		
Chloramphenicol C 30 mcg		21-27 mm		
Gentamicin GEN 10mcg		19-26 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		23-29 mm		
Sulphafurazole SF 300 mcg		15-23 mm		
Cefotaxime CTX 5 mcg		25-31 mm		
Tigecycline TGC 15mcg		20-27 mm		
Tetracycline TE 30 mcg		18-25 mm		
Amoxicillin- clavulanate AMC 30 mcg		18-24 mm		
Ciprofloxacin CIP 5mcg		29-38 mm		
***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 25923 (00034*)**	luxuriant		34-36°C	16-20 hours
Erythromycin E 15 mcg		22-30 mm		
Linezolid LZ 30 mcg		24-30 mm		
Tetracycline TE 30 mcg		24-30 mm		
Ciprofloxacin CIP 5mcg		22-30 mm		
Amoxyclav(Amoxicillin/ Clavulanic acid) AMC 30 mcg		28-36 mm		
Co-Trimoxazole COT 25 mcg		24-32 mm		
Cefoxitin CX 30 mcg		23-29 mm		
Oxacillin OX 1mcg		18-24 mm		
Pristinomycin RP 15 mcg		21-28 mm		
Gentamicin GEN 10 mcg		19-27 mm		
Penicillin-G 10 units		26-37 mm		
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm		
***Staphylococcus aureus* subsp. *aureus* ATCC 29213 (00131*)**	luxuriant		34-36°C	16-20 hours
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. *aureus*ATCC 43300 (MRSA) (00211*)**	luxuriant		34-36°C	24 hours
Oxacillin OX 1 mcg		Very Hazy to No Zone		
Cefoxitin CX 30 mcg		<=21 mm		
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		34-36°C	16-20 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		25-33 mm		
Piperacillin Tazobactum PIT 30/6 mcg		23-29 mm		
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		34-36°C	16-20 hours
Trimethoprim TR 5 mcg #		24-32 mm		
Ampicillin AMP 2 mcg		15-21 mm		
Imipenem IPM 10 mcg		24-30 mm		
Linezolid LZ 10 mcg		19-25 mm		
Nitrofurantoin NIT 100 mcg		18-24 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		26-34 mm		
Vancomycin VA 5 mcg		10-16 mm		
***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 (13,14).

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17. 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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IVD ***In vitro* diagnostic medical device**

CE Marking

20°C – 30°C **Storage temperature**

Do not use if package is damaged

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