Ready Prepared Media

Technical Information

Mueller Hinton Agar Plate (150mm plate)

Product Code: PM 1173CL

Application: 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

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 discthrough an agar gel as described in CLSI Approved Standard (3).

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 (5).

HM infusion B from and Acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starchacts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which servesas 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 (6,7,8). 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 (9)

Type of specimen

Clinical samples - Isolated microorganisms from urine, stool, etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11).

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Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the pack. 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 resultin 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.
7. Each lot of the medium has been tested for the organisms specified on the COA.
8. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

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 (50-100 CFU) aseptically on the plate.

Quality Control

Appearance

Sterile Mueller Hinton Agar 100 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles

Colour of medium

Light amber coloured medium

Quantity of medium

85 ml of medium in 150mm disposable plates.

pH

7.20-7.50

Sterility Check

Passes release criteria

Cultural Response

Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures.

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

Oragnism	Inoculum (CFU)	Standard Zone
Escherichia coli ATCC25922 (00013*)	Luxuriant	

Cephalothin CEP 30mcg		29-37mm
Chloramphenicol C 30 mcg		21-27 mm
Co-Trimoxazole COT 25mcg #		23-29mm
Cefotaxime CTX 30 mcg		29-35 mm
Gentamicin GEN 10 mcg		19-26 mm
Sulphafurazole SF 300 mcg		15-23 mm
Staphylococcus aureussubsp. aureus ATCC 25923 (00034*)	**Luxuriant**	
Co-Trimoxazole COT 25mcg #		# 20 mm (Clear zone)
Cefoxitin CX 30 mcg		23-29 mm
Erythromycin E 15 mcg		22-30 mm
Linezolid LZ 30 mcg		25-32 mm
Oxacillin OX 1mcg		18-24 mm
Pristinomycin RP 15 mcg		21-28 mm
Tetracycline TE 30 mcg $		18-25 mm
Ciprofloxacin CIP 5mcg		22-30 mm
Pseudomonas aeruginosa ATCC 27853 (00025*)	**Luxuriant**	
Ceftazidime CAZ 30 mcg		22-29 mm
Ciprofloxacin CIP 5mcg		30-40 mm
Tobramycin TOB 10 mcg $		19-25 mm
Amikacin AK 30 mcg $		18-26 mm
Aztreonam AT 3mcg		23-29 mm
Cephotaxime CTX 30 mcg		18-22 mm
Gentamicin GEN 10 mcg $		16-21 mm
Imipenem IPM 10 mcg		20-28 mm
Piperacillin PI 100 mcg		12-18 mm
***Escherichia coli* ATCC35218**	**Luxuriant**	
Amoxyclav AMC 30 mcg		18-24 mm
Piperacillin/Tazobactam PIT100/10 mcg		24-30 mm
Ticarcillin TI 75 mcg		6mm
Ticarcillin/Clavulanic acid TCC 75/10mcg		20-28mm
Ampicillin AMP 10 mcg		16-22mm
Ampicillin/Sulbactam A/S10/10 mcg		29-37mm
Enterococcus faecalis ATCC 29212 (00087*)	**Luxuriant**	
Trimethoprim TR 5 mcg #		# 20 mm
Vancomycin VA 30 mcg		17-21mm
Staphylococcus aureus subsp. aureusATCC 43300(MRSA) (00211*)	**Luxuriant**	
Oxacillin OX 1 mcg		Very Hazy to no zone

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 (10,11).

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. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards.
4. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
5. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
6. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.
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. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
10. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
11. 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.

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