Ready Prepared Media

Technical Information

Mueller Hinton Agar plate w/ 5% Sheep Blood

Product Code: PM 2806

Application: Recommended for determination of susceptibility of *Streptococcus* species to antimicrobial agents.

Composition**

Ingredients	Gms / Litre
HM infusion from #	300.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Sheep Blood	50.000 ml

**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). it is further enriched by the addition of sterile blood. Traditionally blood agar bases have incorporated either casein hydrolysate to give rapid production of large colonies or beef infusion to give defined hemolytic reactions. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (2). Mueller Hinton Agar is used as a base for preparing media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives. It supports rapid and luxuriant growth of fastidious and nonfastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Mueller Hinton Agar with 5% sheep blood is recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae.*

Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agargel 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 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* (2). 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 (2,6,7). 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 (8). 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 (8,9).

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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 (3,8,9).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 pack. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handlingspecimens 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. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect thepotency of the disc.
4. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.
5. Each lot of the medium has been tested for the organisms specified on the COA.

Performance and Evaluation

Performace 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 + 5% Sheep Blood in 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles.
Colour
Red coloured medium
Quantity of medium
25ml of medium in disposable plate
Reaction
7.10- 7.50
Sterility test
Passes release criteria
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added 5% w/v sterile sheep blood.

Organism	Growth	Recovery	Haemolysis	Clindamycin CD2 mcg	Erythromycin E 15 mcg	Vancomycin VA 30mcg
Streptococcus pyogenes ATCC 19615	luxuriant	>=70%	beta			
Streptococcus pneumoniae ATCC 49619	luxuriant	>=70%	alpha	19-25mm	25-30mm	20-28mm

Storage and Shelf Life

- On receipt store between 2-8°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 (1,10).

Further Readings

1. 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.
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. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med.,48:330.
8. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa.
9. 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.
10. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

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