Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Mueller Hinton Agar Plate

Product Code: PM 1173

Application: - Mueller Hinton Agar Plate. For the determination of susceptibility of different microorganisms against antimicrobial agents.

Composition**

Ingredients	Gms / Litre
Beef, infusion from 300.000	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

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 [9]. 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]. 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. Beef infusion and casein acid hydrolysate 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 sulfamethoxazoletrimethoprim (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 [1, 2, 6]. 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, 9]. 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 [8].

Methodology

Ready to use sterile poure plates of Mueller Hinton Agar Plate (need no) preparation of media. The media can be used as per the user requirement. These plates are very useful for determination of susceptibility of different microorganisms against different antibiotics by conventional methods; either streak, inoculate or surface spread of the test inoculums (50-100 CFU) aseptically on the plate.

Quality Control

Physical Test

Appearance
Sterile Mueller Hinton Agar in 90 mm disposable Petri plate.
Colour
Light amber coloured medium.
Quantity of medium per Petri plate
25ml of medium in 90 mm plate

Chemical Test

pH 7.20- 7.40

Biological Test

Sterility Testing
Passes release criteria.
Cultural Response
PM 1173: Cultural characteristics observed after incubation at 35-37°C for 18 -24 hours.

Organism	**Growth**
Cultussre Response	
Escherichia coli ATCC 25922	Luxuriant
Enterococcus faecalis ATCC 29212	Luxuriant
Neisseria gonorrhoeae ATCC 49226	Luxuriant
Staphylococcus aureus ATCC 259	Luxuriant
Pseudomonas aeruginosa ATCC 27853	Luxuriant

Storage and Shelf Life

- Store between 15-25°C.
- Use before expiry date as mentioned on the label.
- Don't freeze.

Further Reading

1. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med.<(>,<)>48:330.
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. Cl in. 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. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa.
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams And Wilkins, Baltimore
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. <(>,,<)>

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