

Mueller Hinton Agar Plate**(M700)**

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

Composition

Ingredients	g/ L
Beef infusion.....	2.00
Acid casein peptone.....	17.50
Starch	1.50
Agar.....	17.00

Description

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

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. 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.
- iv. Many data and much experience regarding its performance have been recorded.

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration. WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility. 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*.

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.

Specimen Collection and Handling

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

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Follow good microbiological lab practices while handling specimens and culture.

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**Physical/Chemical control**

Color : Light amber pH: 7.20-7.40

Cultural Response

Cultural characteristics observed after incubation at 35-37°C for 16-20 hours.

Microbiological activity

Organism	Specifications
Escherichia coli ATCC 25922	
Ampicillin 10µg	15-22mm
Pseudomonas aeruginosa ATCC 27853	
Ciprofloxacin 5µg	25-33mm
Staphylococcus aureus ATCC 25923	
Ampicillin 10µg	27-35mm
Trimethoprim sulfamethoxazole 1.25/23.75µg	24-32mm
Staphylococcus aureus ATCC 29213	
Linezolid 10µg	21-27mm
Enterococcus faecalis ATCC 29212	
Trimethoprim sulfamethoxazole 1.25/23.75µg	26-34mm
Vancomycin 5 µg	10-16mm

Storage and Shelf Life

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (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.