

## Technical Information

### Mueller Hinton Agar Plate (200mm plate)

**Product Code: PM 1173XL**

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

#### Composition\*\*

| Ingredients                        | Gms / Litre |
|------------------------------------|-------------|
| 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

#### 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:

- It demonstrates good batch-to-batch reproducibility for susceptible testing.
- It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
- It supports the growth of most non-fastidious bacterial pathogens and
- Many data and much experience regarding its performance have been recorded (4).

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). 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*. HM infusion B from 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). 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 (7,1,2). 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 (4). 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 (4,8). 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). 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 (10).

#### Type of specimen

Clinical samples : Pure cultures isolated from urine , stool, blood etc.



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## Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,10-13). 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.

## Methodology

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

## Quality Control

### Appearance

Sterile Mueller Hinton Agar 200 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles

### Colour of medium

Light amber coloured medium

### Quantity of medium

110ml of medium in 200 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 |
|----------|--------|---------------|------------------------|-------------------|
|----------|--------|---------------|------------------------|-------------------|



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## Ready Prepared Media

|                                                         |           |          |             |
|---------------------------------------------------------|-----------|----------|-------------|
| Escherichia coli ATCC 25922 (00013*)                    | luxuriant | 34-36°C  | 16-20 hours |
| Cephalothin CEP 30mcg                                   |           | 15-21mm  |             |
| Ampicillin AMP 10mcg                                    |           | 15-22mm  |             |
| Chloramphenicol C 30 mcg                                |           | 21-27mm  |             |
| Gentamicin GEN 10mcg                                    |           | 19-26mm  |             |
| Co-Trimoxazole (Sulpha/Trimethoprim) (COT) 25 mcg       |           | 23-29mm  |             |
| Sulphafurazole SF 300 mcg                               |           | 15-23mm  |             |
| Cefotaxime CTX 5 mcg                                    |           | 25-31mm  |             |
| Tigecycline TGC 15mcg                                   |           | 20-27mm  |             |
| Tetracycline TE 30 mcg                                  |           | 18-25mm  |             |
| Amoxicillin- clavulanate AMC 30 mcg                     |           | 18-24mm  |             |
| Ciprofloxacin CIP 5mcg                                  |           | 29-38mm  |             |
| 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                                   |           | 22-30mm  |             |
| Linezolid LZ 30 mcg                                     |           | 24-30mm  |             |
| Tetracycline TE 30 mcg                                  |           | 24-30mm  |             |
| Ciprofloxacin CIP 5mcg                                  |           | 22-30mm  |             |
| Amoxycylav(Amoxicillin/Clavulanic acid) AMC 30 mcg      |           | 28-36mm  |             |
| Co-Trimoxazole COT 25 mcg                               |           | 24-32mm  |             |
| Cefoxitin CX 30 mcg                                     |           | 23-29mm  |             |
| Oxacillin OX 1mcg                                       |           | 18-24mm  |             |
| Pristinomyacin RP 15 mcg                                |           | 21-28mm  |             |
| Gentamicin GEN 10 mcg                                   |           | 19-27mm  |             |
| Penicillin-G 10 units                                   |           | 26-37mm  |             |
| Ampicillin/Sulbactam A/S 10/10 mcg                      |           | 29-37mm  |             |
| 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                                   | luxuriant | 34-36°C  | 24 hours    |





Ready Prepared Media

|                                                       |           |                                   |         |             |
|-------------------------------------------------------|-----------|-----------------------------------|---------|-------------|
| subsp. AureusATCC                                     |           |                                   |         |             |
| 43300 (MRSA) (00211*)                                 |           |                                   |         |             |
| Oxacillin OX 1 mcg                                    |           | Very Hazy to<br>No Zone<br>≤21 mm |         |             |
| Cefoxitin CX 30 mcg                                   |           |                                   |         |             |
| Pseudomonas aeruginosa                                | luxuriant |                                   | 34-36°C | 16-20 hours |
| ATCC 27853 (00025*)                                   |           |                                   |         |             |
| 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                          |         |             |
| Cephataxime CTX 30 mcg                                |           | 18-22 mm                          |         |             |
| Gentamicin GEN 10 mcg \$                              |           | 17-23 mm                          |         |             |
| Imipenem IPM 10 mcg                                   |           | 20-28 mm                          |         |             |
| Piperacillin PI 100 mcg                               |           | 25-33mm                           |         |             |
| Piperacillin Tazobactum                               |           | 23-29 mm                          |         |             |
| PIT 30/6 mcg                                          |           |                                   |         |             |
| Enterococcus faecalis                                 | luxuriant |                                   | 34-36°C | 16-20 hours |
| ATCC 29212 (00087*)                                   |           |                                   |         |             |
| Trimethoprim TR 5 mcg #                               |           | 24-32 mm                          |         |             |
| Ampicillin AMP 2 mcg                                  |           | 15-21mm                           |         |             |
| Imipenem IPM 10 mcg                                   |           | 24-30mm                           |         |             |
| Linezolid LZ 10 mcg                                   |           | 19-25mm                           |         |             |
| Nitrofurantoin NIT 100 mcg                            |           | 18-24mm                           |         |             |
| Co-Trimoxazole (Sulpha/<br>Trimethoprim) (COT) 25 mcg |           | 26-34mm                           |         |             |
| Vancomycin VA 5 mcg                                   |           | 10-16mm                           |         |             |
| Enterococcus faecalis                                 | luxuriant |                                   | 34-36°C | 16-20 hours |
| ATCC33186 (00210*)                                    |           |                                   |         |             |
| Co-Trimoxazole (Sulpha/<br>Trimethoprim) (COT) 25 mcg |           | ≤20 mm                            |         |             |
| Key : *Corresponding WDCM numbers.                    |           |                                   |         |             |

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 (3,5).

## Further Reading

1. Murray P. R., Baron J. H., Tenover F. C. 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., Tenover F. C., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.





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7. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.
8. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobial disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards.
9. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
10. Performance Standards of Antimicrobial Susceptibility Testing; 34th Edition. M100-Ed34, Vol.44, No.5, Jan-2024.
11. ISO/TS 16782:2016, Confirmed in 2021 Clinical laboratory testing - Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing
12. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 14.0, valid from 2024-01-01.
13. European Committee on Antimicrobial Susceptibility Testing Routine and extended internal quality control as recommended by EUCAST Version 14.0, valid from 2024-01-01.

### Disclaimer :

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