Ready Prepared Media

# Technical Information

## Mueller Hinton Agar Plate w/ 2% NaCl

## Product Code: PM 6389

**Application:** Recommended for testing susceptibility of oxacillin against *Staphylococcus* species from clinical samples.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| HM infusion B from # | 300.000 |
| Acicase ## | 17.500 |
| Starch | 1.500 |
| Agar | 17.000 |
| NaCl | 20.00 |
| Final pH ( at 25°C) | 7.4±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 disc through 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. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, whichserves 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 (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 placedon 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 susceptibilitytests, 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).

Mueller Hinton Agar w/2% NaCl isrecommended by CLSI for testing of Oxacillin sensitivity.

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## Type of specimen

Clinical samples : Isolated microorganism from respiratory exudates, urine, stool etc .

## Specimen Collection and Handling

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

## 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 specimensand 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 the potency of the disc.
4. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.
5. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
6. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
7. It is recommended to store the plates at 24-30°C to avoid 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.

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## Quality Control

**Appearance**

Sterile Mueller Hinton Agar Plate w/ 2% NaCl 90 mm disposable plates with smooth surface and absence of black particles/ cracks/bubbles

**Colour of medium**

Light amber coloured medium

**Quantity of medium**

25 ml of medium in 90 mm disposable plates.

**pH**

7.20-7.50

**Sterility Check**

Passes release criteria

**Cultural Response**

Cultural characteristics observed after incubation at 35-37°C for 24 hours. Zone of inhibition in form of ellipse is observed for Oxacillin Ezy MIC™ Strip (EM065) after incubation at 35-37°C for 24 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Observed MIC (µg/ml) Oxacillin Ezy MIC Strip (EM065) |
|---|---|---|---|---|
| *Staphylococcus aureus subsp aureus* ATCC 29213 (00131*) | 50-100 | luxuriant | >=70% | 0.380 |
| *Staphylococcus aureussubsp. aureus* ATCC 43300 (MRSA) (00211*) | 50-100 | luxuriant | >=70% | 64 |

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

## Further Reading


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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.
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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.

## Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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