

**Mueller Hinton Agar**

A solid medium originally designed for the isolation of pathogenic Neisseria species, now widely used for antibiotic susceptibility testing (including sulfonamides).

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| REF: V.1/MU01.100.0100 100 GramREF: V.1/MU01.250.0500 250 Gram | REF: V.1/MU01.500.0500 500 Gram |

# CLINICAL SIGNIFICANCE

# Mueller and Hinton developed Mueller Hinton Agar (MHA) in 1941 for the isolation of pathogenic Neisseria species. Currently, it is recommended by the Clinical and Laboratory Standards Institute (CLSI) for routine susceptibility testing of non-fastidious pathogenic microorganisms by the Kirby-Bauer disk diffusion technique. MHA with 5% sheep blood and MHA with Hemoglobin have been recommended for antimicrobial susceptibility testing of Streptococcus pneumoniae and Haemophilus influenza.

# METHOD PRINCIPLE

MHA contains beef extract and acid hydrolysate of casein which supply amino acids, nitrogenous substances, vitamins, and minerals necessary for microbial growth. Starch is added as a protective agent against toxic materials that may be present in the medium. Also, Starch hydrolysis yields dextrose, which serves as a source of energy. MHA contains low levels of thymidine and thymine, as excess amounts can reverse the inhibitory effect of sulfonamides and trimethoprim. Calcium and magnesium levels are adjusted so that appropriate activity of aminoglycosides, tetracycline and colistin can be expected Pseudomonas aeruginosa. Agar is added as a solidifying agent. Sheep blood may be added to favor the growth of Streptococcus pneumoniae. The Kerby-Bauer procedure requires the addition of a single disc with a certain concentration of antimicrobial agent and then the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values. A certain inoculum of the microorganism is swabbed over the entire surface of the medium. Paper discs impregnated with specific concentrations 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. There are various factors which influence disc diffusion susceptibility testing, like: agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms.

# MEDIA COMPOSITION

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| **Ingredient**  | **Concentration (g/l)** |
| Casein hydrolysate  | 17.5.000 |
| Beef infusion solids | 2.000 |
| Starch  | 1.500 |
| Agar | 15.000 |

## Final pH 7.3 ± 0.2 at 25°C.

**PRECAUTIONS AND WARNINGS**

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

* Wearing personnel protective equipment (overall, gloves, glasses).
* Do not pipette by mouth.
* In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice

immediately.

* Respect country requirement for waste disposal.

***S56:*** dispose of this material and its container at hazardous or special waste collection point.

***S57:*** use appropriate container to avoid environmental contamination.

***S61:*** avoid release in environment.

For further information, refer to the Mueller Hinton Agar material safety data sheet.

# STORAGE AND STABILITY

**Lab.Vie.** Mueller Hinton Agar should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Deterioration

**Lab.Vie.** Mueller Hinton Agar medium is cream to yellow homogeneous free flowing powder. Prepared medium is light amber in color. If there are any physical changes, discard the medium. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations

# SPECIMEN COLLECTION AND PRESERVATION

# For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7, 8). After use, contaminated materials must be sterilized by autoclaving before discarding.

# TYPE OF SPECIMEN

# Clinical samples

# EQUIPMENT REQUIRED NOT PROVIDED

• Sterile cups

• Sterile petri-dishes

• Incubator

• Autoclave

# PROCEDURE

## Suspend 38.0 grams in 1000 ml purified / distilled water.

## Adjust pH to pH 7.3 ± 0.2 at 25°C.

##  Heat to boiling to dissolve the medium completely.

## Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

## Mix well and pour into sterile Petri plates.

# PERFORMANCE CHARACTERISTICS

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

## To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact Lab.Vie. Technical support.

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# RESULTS AND INTERPRETATION

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| **Organisms** | **Growth** | **Antibiotics**  | **Inhibition zone** |
| *Escherichia coli (ATCC 25922)* | Luxuriant | Cephalothin CEP 30mcg Chloramphenicol C 30 mcg Co-Trimoxazole COT 25 mcg ~ Cefotaxime CTX 30 mcg Gentamicin GEN 10 mcg Sulphafurazole SF 300 mcg | 29 -37 mm 21 -27 mm 23 -29 mm 29 -35 mm 19 -26 mm 15 -23 mm |
| *Staphylococcus aureus subsp. aureus (ATCC 25923)* | Luxuriant | Co-Trimoxazole COT 25 mcg ~ Cefoxitin CX 30 mcg Erythromycin E 15 mcg Linezolid LZ 30 mcg Oxacillin OX 1mcg Pristinomycin RP 15 mcg Tetracycline TE 30 mcg \* Ciprofloxacin CIP 5mcg | > 20mm 23-29 mm 22-30 mm 25-32 mm 18-24 mm 21-28 mm 18-25 mm 22-30 mm |
| *Pseudomonas aeruginosa (ATCC 9027)* | Luxuriant | Ceftazidime CAZ 30 mcg Ciprofloxacin CIP 5mcg Tobramycin TOB 10 mcg \* Amikacin AK 30 mcg \* Aztreonam AT 3mcg Cephotaxime CTX 30 mcg Gentamicin GEN 10 mcg \* Imipenem IPM 10 mcg Piperacillin PI 100 mcg | 22-29 mm 30-40 mm 19-25 mm 18-26 mm 23-29 mm 18-22 mm 16-21 mm 20-28 mm 25-33 mm |
| *Pseudomonas aeruginosa (ATCC 9027)* | Luxuriant | Amoxyclav AMC 30 mcg Piperacillin/Tazobactam PIT 100/10 mcg Ticarcillin TI 75 mcg Ticarcillin/Clavulanic acid TCC 75/10mcg Ampicillin AMP 10 mcg Ampicillin/Sulbactam A/S 10/10 mcg | 8-24 mm 24- 30 mm 6 mm 20-28 mm 16-22 mm 29-37 mm |
| *Enterococcus faecalis ATCC 29212* | Luxuriant | imethoprim TR 5 mcg ~ Vancomycin VA 30 mcg | > 20 mm 17-21 mm |
| *Staphylococcus aureus subsp. Aureus (ATCC 43300) (MRSA)* | Luxuriant | Oxacillin OX 1 mcg | No zone |

# RERREFERENCES

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# 2. 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.

# 3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-IdentificationMaintenance of Medical Bacteria, Vol. 1, Williams and Wilkins,

# 4. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa

# 5. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed.,National Committee for Clin. Lab. Standards

# 6. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards

# 7. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards

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| SYMBOLS IN PRODUCT LABELLING |
| IVD | For in-vitro diagnostic use | Number of <n> test in the pack |
| LOT | Batch Code/Lot number | Caution |
| REF | Catalogue Number | Do not use if package isdamaged |
|  | Temperature Limitation |  Consult Instruction for use |
|  | Expiration Date |  |
|  | Manufactured by |  |

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