ARENA BioScien

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Mueller Hinton Agar (MHA)

Solid medium recommended for the use in qualitative procedures to determine the susceptibility of microorganisms to antimicrobial agents

REF: BS.1/MU01.100.0100	100 Gram	REF: BS.1/MU01.250.0250	250 Gram
REF: BS.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

Item	Formula per liter of medium
Casein hydrolysate	17.5 gm
Beef infusion solids	2 gm
Starch	1.5 gm
Agar	17 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

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

BioScien 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.

Final pH 7.3 ± 0.2 at 25°C

PREPARATION

Suspend 38 grams in 1 liter of distilled water, bring to the boil to dissolve the medium completely and sterilize by autoclaving at 121°C for 15 minutes.

Deterioration

The color of ***BioScien*** Mueller Hinton Agar is cream to yellow homogeneous free flowing powder. Prepared Medium, is light amber in color. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

All Clinical specimens that require sensitivity test only.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile petri dishes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Microorganism	Growth	Antibiotics	Zone of inhibition
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
Escherichia coli (ATCC 35218)	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	18-24 mm 24- 30 mm 6 mm 20-28 mm 16-22 mm 29-37 mm
Enterococcus faecalis ATCC 29212	Luxuriant	Trimethoprim 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

***:** The zones for these discs are indicative of the divalent cation content of the medium.
~: The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact ***BioScien*** technical support.

REFERENCE

1. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.
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-Identification-Maintenance 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

SYMBOLS IN PRODUCT LABELLING

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

EC REP

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