

CM 22322 - MUELLER HINTON AGAR PLATE

INTENDED USE

For determination of susceptibility of microorganisms to antimicrobial agents.

PRODUCT SUMMARY AND EXPLANATION

Mueller Hinton agar is used for cultivation of *Neisseria* & for determination of susceptibility of microorganisms to antibiotics. It is formulated by Mueller and Hinton for the primary isolation of *Neisseria* species. Bauer and Kirby recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration. It has become the standard medium for antimicrobial susceptibility testing and its performance is in accordance to Clinical and Laboratory Standard Institute (CLSI), formerly NCCLS and complies with requirements of the WHO, FDA and EUCAST. 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 and
- (iv) Many data and much experience regarding its performance have been recorded.

WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility.

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. 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. 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.

COMPOSITION

Ingredients	Gms / Ltr
Agar	17.000
Casein acid hydrolysate	17.500
Beef, infusion	2.000
Starch	1.500

PRINCIPLE

The medium consists of Beef infusions and Casein acid hydrolysate which provides nitrogen, vitamins, carbon, and amino acids. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. The thymine/thymidine content of this medium is minimized (determined by disc diffusion procedure with *Enterococcus faecalis* ATCC 29212 and sulfamethoxazole-trimethoprim antibiotic) and levels of calcium and magnesium are adjusted (determined by *Pseudomonas aeruginosa* ATCC 27853 and aminoglycoside antibiotics) to give consistent zones of inhibition as per specified diameters in the CLSI standards.

INSTRUCTION FOR USE

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



QUALITY CONTROL SPECIFICATIONS

Appearance	:	Light amber colour, clear to slightly opalescent gel
Quantity of Medium	:	25ml of medium in 90mm plates.
pH (at 25°C)	:	7.3± 0.2
Sterility Check	:	Passes release criteria

INTERPRETATION

Growth Promotion test

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
<i>Staphylococcus aureus</i>	25923	50-100	Luxuriant	≥70%	30-35°C	18-24 hours
<i>Escherichia coli</i>	25922	50-100	Luxuriant	≥70%	30-35°C	18-24 hours
<i>Pseudomonas aeruginosa</i>	27853	50-100	Luxuriant	≥70%	30-35°C	18-24 hours
<i>Enterococcus faecalis</i>	29212	50-100	Luxuriant	≥70%	30-35°C	18-24 hours

Antibiotic Susceptibility Test

Cultural characteristics observed after inoculating 0.5 McFarland culture (1-2 x 10⁸ CFU/ml) by lawn technique, dispensing antibiotic discs and incubation at 30-35°C for 18 hours. After incubation, inhibition zone diameter measured in mm.

Antibiotics	<i>Escherichia coli</i> (ATCC 25922)	<i>Staphylococcus aureus</i> (ATCC 25923)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	<i>Enterococcus faecalis</i> (ATCC 29212)
	Std.	Std.	Std.	Std.
Amoxicillin (10 mcg)	19-25	28-36	Resistant	-
Ampicillin (10 mcg)	15-22	27-35	Resistant	-
Amoxiclavate (10mcg)	18-24	28-36	Resistant	-
Amikacin (30 mcg)	19 - 26	20 - 26	18 - 26	-
Azithromycin (15 mcg)	Resistant	21 - 26	Resistant	-
Cefotaxime	29-35	25-31	18- 22	-
Colistin (Methane Sulphate) (10 mcg)	11 - 17	Resistant	11 - 17	-
Cefaclor (30 mcg)	23 - 27	27 - 31	Resistant	-
Cefoperazone (75 mcg)	28 - 34	24 - 33	23 - 29	-
Cefazolin (30 mcg)	21 - 27	29 - 35	Resistant	-
Cefuroxime (30 mcg)	20 - 26	27 - 35	Resistant	-
Cephalothin (30 mcg)	15 - 21	29 - 37	Resistant	-
Chloramphenicol (30 mcg)	21 - 27	19 - 26	Resistant	-
Ciprofloxacin (5 mcg)	29-37	22 - 30	25 - 33	-
Erythromycin (15 mcg)	Resistant	22 - 30	Resistant	-
Gentamicin (10 mcg)	19 - 26	19 - 27	17 - 23	-



Kanamycin (30 mcg)	17-25	19 - 26	Resistant	-
Norfloxacin (10 mcg)	28 - 35	17 - 28	22 - 29	-
Lomefloxacin (10 mcg)	27 - 33	23 - 29	22 - 28	-
Co-Trimoxazole (25 mcg)	23-29	24-32	Resistant	≥ 20

PACKAGING:

Doubled layered packing containing 5 No. of plates with one silica gel desiccant bag packed inside it.

STORAGE

On receipt, store the plates at 15–30 °C. Avoid freezing and overheating. Do not open until ready to use. Prepared plates stored in their original sleeve wrapping until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

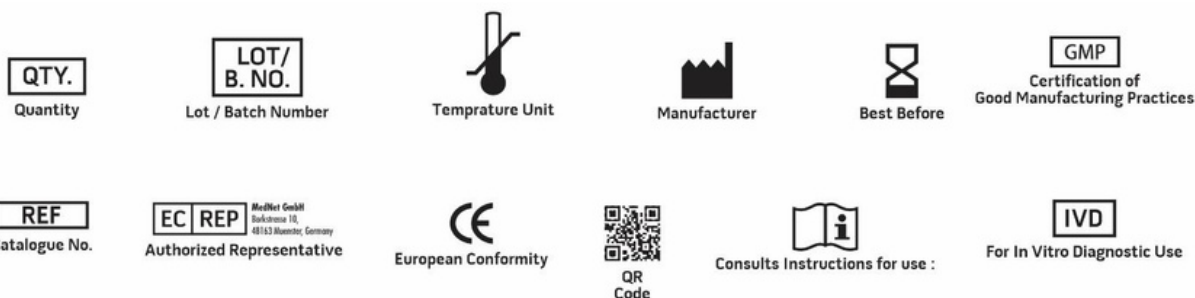
Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- Mueller, J.H. and Hinton, J. 1941. Proc. Soc. Exp. Biol. Med. 48: 3330-3333.
- Gordon, M.H. and Hine, T.G.M. 1916. Br. Med. J. 18: 678-684.
- Bauer, A.L., Kirby, W.M.M., Sherris, J.C., Turck, M. 1966. Am. J. Clin. Pathol. 45: 493-496.
- World Health Organization. 1961. Standardization of methods for conducting microbial sensitivity tests. Technical Report Series No. 210, Geneva.
- Food and Drug Administration. 1998. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
- Wood, G.L. and Washington, J.A. 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods, p. 1327-1341. In Murray, P.R., Baron, E.J., Tenover, F.C. and Tenover R.H. (Eds.). Manual of clinical microbiology, 6th ed., American Society for Microbiology, Washington, D.C.
- National Committee for Clinical Laboratory Standards. 1997. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA.
- Clinical and Laboratory Standards Institute (formerly NCCLS). 2006. Performance standards for antimicrobial disk susceptibility tests; approved standard, 9th ed. Clinical and Laboratory Standards Institute document M2-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2013. Standards for Antimicrobial Susceptibility Testing; Twenty Third Informational Supplement, M100-S23 (MS). Wayne, PA.
- Matuschek, E., Brown, D.F.J. and Kahlmeter, G. 2014. Clin. Microbiol. Infect. 20: O255–O266.
- The European Committee on Antimicrobial Susceptibility Testing. 2014. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing, version 4.0



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For LabUse Only**

