Mueller Hinton Agar

Intended Use

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

Typical Composition (g/litre)

HM infusion B 300.0; casein hydrolysate17.5; Starch 1.50; Agar 17.0

Mode of Action

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic Neisseria species.

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

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration. WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility. 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*.

Preparation

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. The plates are clear to opalescent and yellowish brown.

Final pH (at 25°C) 7.3±0.1

Storage

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Specimen

Clinical samples: Isolated microorganisms from urine, stool, blood etc.

Experimental Procedure and Evaluation

Carry out the sensitivity or resistance test as directed. Incubation for 24 h at 35 °C aerobically.

Quality Control

Organism	Growth	Standard zone of inhibition	Observed
Escherichia coli ATCC 25922	Luxuriant		
Cephalothin 30mcg		29-37 mm	29-37 mm
Chloramphenicol 30 mcg		21-27 mm	21-27 mm
Co-Trimoxazole 25 mcg		23-29 mm	23-29 mm
Cefotaxime 30 mcg		29-35 mm	29-35 mm
Gentamicin 10 mcg		19-26 mm	19-26 mm
Sulphafurazole 300 mcg		15-23 mm	15-23 mm
Staphylococcus aureus ATCC 25923	Luxuriant		
Co-Trimoxazole 25 mcg		20-20 mm	20-20 mm
Cefoxitin 30 mcg		29-37 mm	29-37 mm
Erythromycin 15 mcg		22-30 mm	22-30 mm
Linezolid 30 mcg		25-32 mm	25-32 mm
Oxacillin 1mcg		18-24 mm	18-24 mm
Pristinomycin 15 mcg		21-28 mm	21-28 mm
Tetracycline 30 mcg		18-25 mm	18-25 mm
Ciprofloxacin 5mcg		22-30 mm	22-30 mm
Pseudomonas aeruginosa ATCC 27853	Luxuriant		
Ceftazidime 30 mcg		22-29 mm	22-29 mm
Ciprofloxacin 5mcg		30-40 mm	30-40 mm
Tobramycin 10 mcg		19-25 mm	19-25 mm
Amikacin 30 mcg		18-26 mm	18-26 mm
Aztreonam 3mcg		23-29 mm	23-29 mm
Cephotaxime 30 mcg		18-22 mm	18-22 mm
Gentamicin 10 mcg		16-21 mm	16-21 mm
Imipenem 10 mcg		20-28 mm	20-28 mm

Piperacillin 100 mcg		12-18 mm	25-33 mm
Escherichia coli ATCC 35218	Luxuriant		
Amoxyclav 30 mcg		18-24 mm	18-24 mm
Piperacillin/Tazobactam 100/10 mcg		24-30 mm	24-30 mm
Ticarcillin 75 mcg		6 mm	6 mm
Ticarcillin/Clavulanic acid 75/10 mcg		20-28 mm	20-28 mm
Ampicillin 10 mcg		16-22 mm	16-22 mm
Ampicillin/Sulbactam 10/10 mcg		29-37 mm	29-37 mm
Enterococcus faecalis ATCC 29212	Luxuriant		
Trimethoprim 5 mcg		>=20 mm >=20 mm	>=20 mm >=20 mm
Vancomycin 30 mcg		17-21 mm	17-21 mm
Staphylococcus aureus ATCC 43300	Luxuriant		
Oxacillin 1 mcg		Very Hazy to No zone	No Zone

Reference

1. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol. 45:493.
2. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol. Scand. Sect B Suppl., 217:1.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.