HiEncap™ Mueller Hinton Agar

EC173CCL

HiEncap™ Mueller Hinton Agar is recommended for determination of susceptibility of microorganisms to antimicrobial agents

Composition**

Ingredients	Gms / Litre
Beef, infusion from	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Each capsule contains 9.5 gms of medium. Suspend 1 capsule in 250 ml (4 capsules in 1000 ml)distilled or purified water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring. Note: The performance of this batch has been tested and standardized as per the current CLSI (formerly,NCCLS) document M6-Protocols for Evaluating Dehydrated Mueller Hinton Agar.

Principle And 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). 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 (9).

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). 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* .

Beef infusion and casein acid hydrolysate 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, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazoletrimethoprim (SXT). Calcium and magnesium ion concentrations are adjusted to provide the amounts recommended by CLSI to give the correct MIC values with aminoglycosides and *Pseudomonas aeruginosa* (2).

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 (1, 2, 6). 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 (7). 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 (7, 9).

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 (8).

Quality Control

Appearance
Gelatin capsule containing cream to yellow coloured granular media

Quantity
Each capsule contains 9.5 grams of medium sufficient for 250 ml medium

Gelling
Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium
Light amber coloured clear to slight opalscent gel froms in Petri plates.

Reaction
Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH
7.20-7.40

Cultural Response
Cultural characteristics observed after incubation at 35-37°C for 18 -24 hours

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922	50 -100	luxuriant	>=70 %
Haemophilus influenzae ATCC 49247	50 -100	good-luxuriant (on Mueller Hinton Chocolate Agar)	>=70%
Neisseria gonorrhoeae ATCC 49226	50 -100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853	50 -100	luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212	50-100	luxuriant	>=70%
Streptococcus pneumoniae ATCC 6305	50-100	luxuriant (on Mueller Hinton Blood Agar)	>=70%
Escherichia coli ATCC 35218	50 -100	luxuriant	>=70 %
Staphylococcus aureus ATCC 43300	50-100	luxuriant	>=70%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C.Use before expiry date on the label.

Reference

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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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9. 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.

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