

70191 Mueller Hinton Agar (M-H Agar)

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

Composition:

Ingredients	Grams/Litre
Beef infusion solids	2.0
Starch	1.5
Casein hydrolysate	17.5
Agar	17.0
Final pH 7.3 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions :

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

Principle and Interpretation:

Mueller Hinton Agar is recommended for the disk diffusion method of antibiotic susceptibility testing. Mueller Hinton medium is recommended by FDA, World Health Organization and NCCLS for testing most commonly encountered aerobic and facultative anaerobic bacteria in food and clinical material. The medium shows good batch-to-batch reproducibility. It is low in sulfonamide, trimethoprim, and tetracycline inhibitors and yields satisfactory growth of most non-fastidious pathogens. Beef infusion and Casein provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Mueller Hinton media. Starch is added to absorb any toxic metabolites produced. Paper discs impregnated with certain amount of specific antibiotics are placed on the surface of the medium. The plates are incubated and the zones of inhibition around each disc are measured. Different factors influence the disc diffusion susceptibility tests as inoculum concentration, agar depth, disc potency, medium pH and beta-lactamase production by test organisms. For testing streptococci, supplementation with 5% defibrinated sheep or horse blood is recommended. Mueller Hinton media should be supplemented with 2% NaCl for testing methicillin or oxacillin (28221) against staphylococci. Mueller Hinton media with Rabbit Serum is used for the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim is demonstrated on medium possessing high levels of thymidine. The PABA and thymine/thymidine content of Mueller Hinton media is reduced to a minimum.

Cultural characteristics after 24 hours at 35°C.

Organisms (ATCC)	Growth
<i>Escherichia coli</i> (25922)	+++
<i>Staphylococcus aureus</i> (25923)	+++
<i>Pseudomonas aeruginosa</i> (27853)	+++
<i>Neisseria meningitidis</i> (13090)	+++
<i>Streptococcus faecalis</i> (29212)	+++



References:

1. NCCLS Approved Standard: ASM-2, Performance Standards for Antimicrobial disc Susceptibility Tests, 2nd ed., National Committee for Clin. Lab. Standards (1979)
2. NCCLS M7-T Tentative Standard, National Committee for Laboratory Standards (1983)
3. Bauer et al, Am. J. Clin. Path., 45, 493 (1966)
4. Ericsson and Sherris, Acta. Pathol. Microbiol., Scand. Sec. B. Suppl., 217, 1 (1971)
5. Present Status and Future Work, WHO Sponsored collaborative study, Chicago (Oct. 1967)
6. J.H. Müller, J. Hinton, Proc. Soc. Exptl. Biol. 48, 330 (1941)
7. G.M. Eliopoulos, et al., Enhancement of cefotaxime and other cephalosporins against *Enterococcus faecalis* by blood supplemented Mueller-Hinton agar, Diagn. Microbiol. Infect. Dis. 12, 149 (1989);
8. R.D. Jenkins, et al., J. Clin. Microbiol. 22, 369 (1985)

