Mueller Hinton Broth

Intended Use

Mueller Hinton Broth is recommended to determine the susceptibility of bacteria to sulphonamides by the tube dilution method.

Typical Composition (g/litre)

Beef infusion 300.0 ; Casein acid hydrolysate17.5 ; Starch 1.50

Description

Studies on antibiotic susceptibilities are being made both in broth and agar, it will be found to be of particular value to have media of identical nutrient formulation. Mueller-Hinton Broth is recommended for broth dilution MIC studies* of all species of most commonly encountered aerobic and facultatively anaerobic bacteria.

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.

If MIC is being done Mueller-Hinton Broth will require supplementation with the divalent cations Mg++ and Ca++ after sterilisation. The CLSI recommend the following cation levels Ca++, 20-25mg/litre; Mg++, 10-12.5mg/litre.

Preparation

Suspend 21.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely.

Mix well and dispense into tubes as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool at room temperature and adjust cation levels if necessary*

Make stock solution of Ca2+ by adding 8.36g of MgCl2.6H2O into 100ml H2O and sterile filter solution resulting a final concentration of 10mg/ml.

Make stock solution of Mg2+ by adding 3.68g of CaCl2.2H2O into 100ml H2O and sterile filter solution resulting a final concentration of 10mg/ml.

Add 1ml of Mg+2 stock and 2ml of Ca+2 to prepared media as a supplement for MIC studies.

Final pH (at 25°C) 7.3±0.1

Storage

Store DCM between 10-30°C in a tightly closed container and Use before expiry date on the label.

Quality Control

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922	50-100	Good-Luxuriant
Neisseria gonorrhoeae ATCC 49226	50-100	Good-Luxuriant
Staphylococcus aureus ATCC 25923	50-100	Good-Luxuriant
Pseudomonas aeruginosa ATCC 27853	50-100	Good-Luxuriant
Enterococcus faecalis ATCC 19433	50-100	Good-Luxuriant
Streptococcus pneumonia ATCC 6305	50-100	Good-Luxuriant
Haemophilus influenza ATCC49247	50-100	Good-Luxuriant

Reference

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2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
4. National Committee for Clinical Laboratory Standards (2000) *Methods for Dilution Antimicrobial Susceptibility Tests for bacteria that grow aerobically. Approved Standard M7-A5. NCCLS. Villanova, Pa*
5. Thornsberry C., Gavan T. L. and Gerlach E. H. (1977) *Cumitech 6. American Society for Microbiology. Washington DC.*