

CM 21244 - MUELLER HINTON BROTH

INTENDED USE

For testing the susceptibility of microorganisms to sulphonamides by tube dilution method.

PRODUCT SUMMARY AND EXPLANATION

Mueller Hinton Broth is a stable serum-free bacteriological media that supported the growth of two otherwise very fastidious bacteria, the meningococcus and the gonococcus. Later it was improved by supplementing cations to enhance Pseudomonas recovery and for propagating the vast majority of pathogenic bacteria encountered in clinical practice. In the beginning of the antibiotic era, the versatility of Mueller Hinton Broth and its agar was a perfect match, allowing a standardized, consistent medium for broth dilution or disk diffusion testing of antibiotic potency, defined most frequently in terms of minimum inhibitory concentration (MIC). In recent decades, MHB has been endorsed by the Clinical and Laboratory Standards Institute (CLSI), the global nonprofit organization that ensures quality in healthcare testing, as the appropriate media for routine bacterial antibiotic susceptibility determination, with updated cutoff standards for designating resistant and susceptible strains.

COMPOSITION

Ingredients	Gms / Ltr
Beef, infusion form	300.000
Casein acid hydrolysate	17.500
Starch	1.500

PRINCIPLE

Beef, infusion form 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. These ingredients are selected for low thymine and thymidine content as determined by MIC values for Enterococcus faecalis with sulfamethoxazole trimethoprim (SXT). The CLSI recommend the following cation levels Ca⁺⁺, 20-25mg/liter; Mg⁺⁺, 10-12.5mg/liter to be present in the broth while performing broth dilution method. Cation level is usually inadequate in the medium; the concentration of cations already present in the broth must be the amount of Ca⁺⁺ or Mg⁺⁺; if that needs to be added. Cation stock for addition of supplemental cations are recommended by CLSI which is 8.36g of MgCl₂.6H₂O in 100ml contains 10mg of Mg⁺⁺/ ml and 3.68g of CaCl₂.2H₂O 100ml contains 10 mg of Ca⁺⁺/ml. Alternatively, Mueller Hinton Broth No. 2 Control Cations is available wherein cations are adjusted as per Standard guidelines.

INSTRUCTION FOR USE

- Dissolve 21.0 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Mix well and dispense into tubes or flasks as desired.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

Note: It is suggested to boil the medium before autoclaving to avoid settling of starch at the bottom.

QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder : Cream to yellow homogeneous free flowing powder.
- Appearance of prepared medium : Light amber coloured clear solution in tubes.
- pH (at 25°C) : 7.3±0.2

INTERPRETATION



Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Good-luxuriant	35-37°C	18-24 Hours
Pseudomonas aeruginosa	27853	50-100	Good-luxuriant	35-37°C	18-24 Hours
Staphylococcus subsp. aureus	25923	50-100	Good-luxuriant	35-37°C	18-24 Hours
Enterococcus faecalis	19433	50-100	Good-luxuriant	35-37°C	18-24 Hours

PACKAGING:

Inpacksize of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

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

DISPOSAL

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

REFERENCES

1. CLSI: Clinical and Laboratory Standards Institute. 25th Informational Supplement. Clinical and Laboratory Standards Institute; Wayne, PA: 2015. Performance standards for antimicrobial susceptibility testing; p. M100-S25.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Mueller J.H., Hinton J. A protein-free medium for primary isolation of the Gonococcus and Meningococcus. Proc. Soc. Exp. Biol. Med. 1941;48:330-333.
5. Victor Nizet; "The Accidental Orthodoxy of Drs. Mueller and Hinton"; EBioMedicine 22 (2017) 26-27 PMID:28689739.

