
Mueller Hinton Broth No.2 Control Cations

Intended Use

Mueller Hinton Broth No.2 Control Cations is intended for use in quantitative procedures for susceptibility testing of rapidly growing aerobic and facultatively anaerobic bacteria isolated from clinical specimens.

Typical Composition (g/litre)

Beef extract 3.0 ; Casein acid hydrolysate 17.50 ; Starch 1.50

Mode of Action

Mueller Hinton Broth No. 2 Control Cations is used in the susceptibility testing of rapidly growing aerobic and facultatively anaerobic bacteria from clinical specimens. The medium is designed to give a low thymine and thymidine content and also the calcium and magnesium ion concentration is adjusted as recommended by CLSI. The medium is not recommended for fastidious organisms. Thymine and thymidine inhibit sulfonamide and trimethoprim activity and calcium and magnesium interferes with the activity of aminoglycoside antibiotics. Beef extract 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 sulfamethoxazole trimethoprim.

Preparation

Suspend 22 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 115-121°C (10-15 lbs pressure respectively) for 10 minutes. Do not over heat. Final pH (at 25°C) 7.3±0.1

Note: This medium is supplemented with appropriate salts to provide 20-25 mg/l of calcium and 10-12.5 mg/l of magnesium and as additionally required to suit performance parameters.

Storage

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

Experimental Procedure and Evaluation

In Mueller Hinton Broth No. 2 Control Cations, antimicrobial agents are prepared in serial two-fold dilutions and are inoculated with the test culture to give a final concentration of 5×10^5 CFU/ml. Following incubation at 35°C; the presence of turbidity indicates growth of the organism. The lowest concentration of antimicrobial

agent showing no growth is the MIC of that organism for that agent. The interpretation as to whether the organism is susceptible, intermediate, or resistant in its response to the agent is made by comparing the MIC to those in the MIC interpretive standards in CLSI standard M7. Various factors have been identified as influencing broth dilution susceptibility tests. These include the medium, antimicrobial potency, inoculum concentration, pH, antimicrobial stability and mechanisms of resistance by the test organisms.

Quality Control

Organism	Inoculum	Growth
Escherichia coli ATCC 25922	50 - 100	Good-Luxuriant
Pseudomonas aeruginosa ATCC 27853	50 - 100	Good-Luxuriant
Staphylococcus aureus ATCC 25923	50 - 100	Good-Luxuriant
Enterococcus faecalis ATCC 29212	50 - 100	Good-Luxuriant

Reference

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5. Thornsberry C., Gavan T. L. and Gerlach E. H., 1977, Cumitech 6, New developments in antimicrobial agent susceptibility testing. Coord. Ed., Sherris. American Society for Microbiology, Washington, D.C.
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