Mueller Hinton Broth No.2 Control Cations

M1657

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.

Composition**

Ingredients	Gms / Litre
Beef extract	3.000
Casein acid hydrolysate	17.500
Starch	1.500
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22 grams in 1000 ml 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 OVERHEAT.

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.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* (1). Development of other media led to the replacement of the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* , but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. It is now used as a test medium for antimicrobial susceptibility testing (2).

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 (2). The medium is not recommended for fastidious organisms. Thymine and thymidine inhibit sulfonamide and trimethoprim (3, 4) activity and calcium and magnesium (5, 6) 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 (SXT).

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 5×105 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 (2, 7). 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 (8- 10).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

Reaction of 2.2% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH
7.20-7.40

Cultural Response
M1657: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours .

Organism	Inoculum (CFU)	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

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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