TM 1577 - MUELLER HINTON BROTH NO.2

INTENDED USE

For quantitative susceptibility testing of rapidly growing aerobic and facultative anaerobic bacteria isolated from clinical specimen.

PRODUCT SUMMARY AND EXPLANATION

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria*. 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.

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.

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. 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.

COMPOSITION

Ingredients	Gms / Ltr
Beef extract	3.000
Casein acid hydrolysate	17.500
Starch	1.500

PRINCIPLE

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.

INSTRUCTION FOR USE

- Dissolve 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 psi 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.

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 aureus	25923	50-100	Good-luxuriant	35-37°C	18-24 Hours
Enterococcus faecalis	29212	50-100	Good-luxuriant	35-37°C	18-24 Hours

PACKAGING:

In pack size 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

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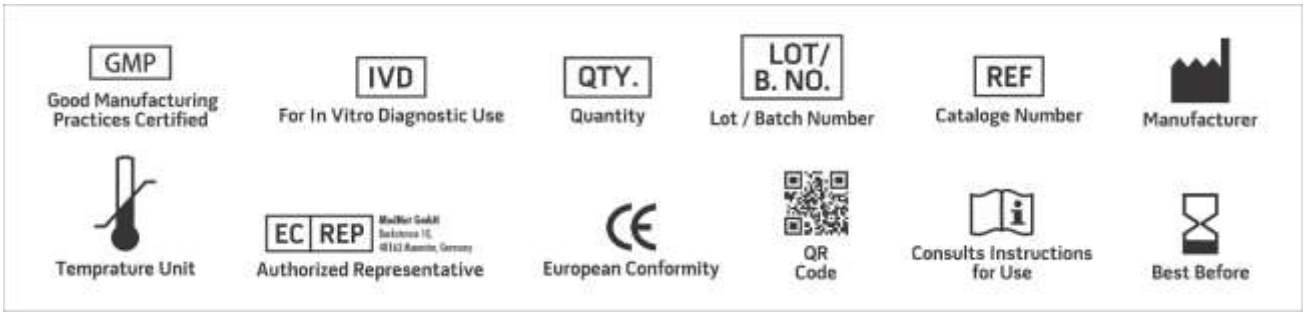

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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