

## Mueller Hinton II Broth w/ Horse Blood

Liquid medium for quantitative antimicrobial susceptibility testing of fastidious organisms by broth dilution procedures.

TYPICAL FORMULA(*)	(g/l)
Beef Extract	3.0
Acid Hydrolysate of Casein	17.5
Starch	1.5
Lysed Horse Blood	20 ml
Final pH 7.3 ± 0.1	

(\*) Adjusted and/or supplemented as required with appropriate salts to provide 20-25 mg/l of calcium and 10-12.5 mg/l of magnesium.

### DESCRIPTION

Mueller Hinton Broth is a liquid medium recommended for quantitative antimicrobial susceptibility testing of common, rapidly growing aerobic and facultatively anaerobic bacteria by broth dilution procedures, as standardized by the Clinical and Laboratory Standard Institute (CLSI).

Mueller Hinton II Broth is formulated to have low levels of thymine and thymidine and is adjusted to the calcium and magnesium ions concentrations recommended in CLSI document M7.

Cation-Adjusted Mueller Hinton Broth (CAMHB) supplemented with 2-5% lysed horse blood (LHB) is the medium recommended for susceptibility testing of streptococci and *N. meningitidis*.

#### **PRINCIPLE**

Acid hydrolysate of casein and beef extract provide amino acids, nitrogen, minerals, vitamins, carbon and other nutrients which support the growth of microorganisms. Starch acts as a protective colloid against toxic molecules which can be present in the medium. Hydrolysis of starch during autoclaving supplies a little amount of glucose, which is a source of energy. Lysed horse blood is included to improve the MIC endpoints of sulphonamides and trimethoprim.

Broth dilution methods, macrodilution and microdilution, are used to measure quantitatively the *in vitro* activity of an antimicrobial agent against a given bacterial isolate. To perform the test, a series of tubes/wells is prepared with a broth medium to which various concentrations of the antimicrobial agents are added. The tubes/wells are then inoculated with a standardized suspension of the test organism. After incubation at  $35 \pm 2^{\circ}$ C, the tests are examined and the MIC (Minimum Inhibitory Concentration) is determined.

#### **TECHNIQUE**

- 1. Prepare antimicrobial agents in serial two-fold dilutions in Mueller Hinton II Broth w/ Horse Blood.
- 2. Prepare a standardized suspension of the test organism using the direct colony suspension method.
- 3. Add the suitable volume of the adjusted inoculum to each tube containing the antimicrobial agent in the dilution series, and mix.
- 4. Incubate the tubes at  $35 \pm 2$ °C for 20-24 hours in ambient air (enriched with 5% CO<sub>2</sub> if N. meningitidis is tested).

#### INTERPRETATION OF RESULTS

After incubation 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.

Interpret the MIC by referring to the current CLSI document M100 and report the organism as susceptible, intermediate or resistant to the agents that have been tested.

### STORAGE

2-8°C away from light, until the expiry date on the label. Eliminate if signs of deterioration or contamination are evident.

#### WARNING AND PRECAUTIONS

For In Vitro Diagnostic use. For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

#### **DISPOSAL OF WASTE**

Disposal of waste must be carried out according to the national and local regulations in force.

#### REFERENCES

- CLSI. Methods for dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Tenth Edition. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
- 2. D'Amato and Thornsberry. 1979. Current Microbiol. 2:135.
- 3. Swenson J. M. and Thornsberry C. 1978. Curr. Microbiol. I. 189-193.
- 4. Pollock, Minshew, Kenny and Schoenknecht. 1978. Antimicrob. Agents Chemother. 14:360.
- 5. Thornsberry, Gavan and Gerlach. 1977. Cumitech 6, New developments in antimicrobial agent susceptibility testing. Coord. ed., Sherris. American Society for Microbiology, Washington, DC.
- 6. Ferone, Bushby, Burchall, Moore and Smith. 1975. Antimicrob. Agents Chemother. 7:91.
- 7. Reller, Schoenknecht, Kenny and Sherris. 1974. J. Infect. Dis. 130:454.
- 8. Koch and Burchall. 1971. Appl. Microbiol. 22:812.
- 9. Ryan, Schoenknecht and Kirby. 1970. Hospital Practice 5:91.
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- 11. Bauer, Kirby, Sherris and Turck. 1966. Am. J. Clin. Pathol. 45:493.







### **PRODUCT SPECIFICATIONS**

#### NAME

Mueller Hinton II Broth w/ Horse Blood

### **PRESENTATION**

Glass tubes containing 11 ml of liquid medium

#### STORAGE

2-8°C

#### **PACKAGING**

7.0.0.0				
Ref.	Content	Packaging		
23002	10 x 11 ml tubes	10 tubes in cardboard box		

# pH OF THE MEDIUM

 $7.3 \pm 0.1$ 

#### USE

Mueller Hinton II Broth w/ Horse Blood is the medium recommended for susceptibility testing of streptococci and N. meningitidis

#### **TECHNIQUE**

Refer to technical sheet of the product

### APPEARANCE OF THE MEDIUM

Red, clear

#### SHELFLIFE

1 year

### **QUALITY CONTROL**

- 1. Control of general characteristics, label and print
- 2. Sterility control

7 days at  $22 \pm 2^{\circ}$ C, in aerobiosis 7 days at  $35 \pm 2^{\circ}$ C, in aerobiosis

Microbiological control

Incubation Conditions: 35 ± 2°C for 20-24 h

Inoculum: ≤10<sup>4</sup> CFU/mI

Microorganism Growth

Streptococcus pneumoniae ATCC® 49619 Good

TABLE OF SYMBOLS										
LOT	Batch code	IVD	In vitro Diagnostic Medical Device	***	Manufacturer	$\square$	Use by	1	Fragile, handle with care	
REF	Catalogue	1	Temperature limitation	Σ	Contains sufficient for <n> tests</n>	[]i	Caution, consult	(2)	) Do not reuse	

