MUELLER – HINTON 69444

BROTH / CULTURE MEDIUM FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

IVD

1- INTENDED USE

Mueller-Hinton broth is a liquid nutritional medium used for antimicrobial susceptibility testing of aerobic and facultative anaerobic bacteria . It is used as a culture medium to perform antimicrobial susceptibility testing according to the dilution method (1, 2) and as a base medium to prepare bacterial inocula.

2- PRINCIPLE

The accuracy of the antimicrobial susceptibility test results is ensured by standardization of the composition of the medium used. Variations of pH, concentrations of divalent cations and/or thymidine can alter the results obtained. Bio-Rad's Mueller-Hinton broth therefore has a low thymine and thymidine concentration and a defined calcium and magnesium concentration. This formulation ensures the accuracy of the minimal inhibitory concentrations (MIC) obtained with the reference strains recommended by international guidelines (4, 5).

3- HOW SUPPLIED

- Dehydrated medium
  - 500 g bottle code **69444**

4- THEORETICAL COMPOSITION (in g/l of distilled water)*

Mueller-Hinton medium is prepared according to the formula described by W.H.O. (6)

Dehydrated meat infusion	2
Casein hydrolysate	17.5
Starch	1.5
Final pH	7.3 ± 0.1
Ca^{2+}	20-25 mg/L
Mg^{2+}	10-12.5 mg/L

* formula adapted and/or supplemented to ensure the best performances of the medium.

Preparation of the medium:
Homogenize the powder contained in the bottle.
Mix **25 grams** of dehydrated medium in 1 litre of freshly distilled water until a homogeneous suspension is obtained. Sterilize in the autoclave at 121°C ± 1°C for 15 minutes. Dispense into sterile tubes or bottles.

5- STORAGE

- Dehydrated medium: tightly sealed bottle in a dry place (+15-25°C).

The expiry date and batch number are indicated on the packaging.

6- INSTRUCTIONS

Material

- Material provided: Mueller-Hinton medium
- Specific material not provided
  - Mac Farland 0.5 opacity standard
  - Laboratory equipment necessary for antimicrobial susceptibility testing by the dilution method.

Precautions for use: Comply with the instructions of current guidelines (CLSI[4], CA-SFM[5]). Always observe current techniques and precautions concerning protection against microbiological hazards.

Inoculation:
From a pure, fresh culture, prepare a suspension in Mueller-Hinton broth with an opacity equivalent to Mac Farland 0.5 standard. The inoculum standardization protocol is described in the various guidelines published by the CLSI and the CA-SFM for the dilution method.

Incubation:
Mueller-Hinton broth can be used to prepare the bacterial inoculum and as a culture medium for determination of MIC.
Refer to the CLSI and/or CA-SFM guidelines for detailed procedures (4, 5).

Interpretation
The interpretation of the antimicrobial susceptibility test using the MIC method is described in periodic updates of the CA-SFM and CLSI guidelines

7- PERFORMANCE/QUALITY CONTROL

- Appearance of the dehydrated medium: **beige** powder.
- The growth performances of Mueller Hinton medium are verified with the following strains:

STRAINS	DILUTION ANTIMICROBIAL SUSCEPTIBILITY TESTING MIC (µg/ml)
Escherichia coli ATCC 25922 - Ampicillin - Tetracycline	MIC complying with CLSI and/or CA-SFM guidelines
Pseudomonas aeruginosa ATCC 27823 - Tobramycin - Ciprofloxacin	
Enterococcus faecalis var *zymogenes* ATCC 29212 - Trimethoprim / Sulfamethoxazole	

8- LIMITS OF USE

- Pure, fresh cultures must always be used to obtain interpretable results.
- Some strains may not grow on this medium due to their nutritional requirements, particularly *Haemophilus*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and β–haemolytic Streptococci. It is therefore recommended to comply with the CLSI and/or CA-SFM guidelines.
- A number of factors can affect the results (size of the inoculum, incubation time and atmosphere, etc.). It is therefore essential to comply with the protocol described in the current guidelines (CLSI, CA-SFM).

9- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

10- REFERENCES

1. MUELLER J.H. and HINTON J. Proc. Soc. Exp. Biol. Med., 1941, **48**, 330-333.
2. OLSENA M. and SCOTT W.J. Nature, 1946, **157**, 337.
3. C.L.S.I.-M7-A. 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard.
4. C.L.S.I.-M32-P. Evaluation of Lots of Dehydrated Mueller-Hinton Broth for Antimicrobial Susceptibility Testing; Proposed Guideline
5. Comité de l'antibiogramme. 2002. Société Française de Microbiologie.
6. World Heath Organization Expert Committee on Biological Standardization. 1981. Technical report series 673 (Revision 1981). W.H.O., Geneva – p156-192.

Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette France
Tel. : +33 (0) 1 47 95 60 00
Fax : +33 (0) 1 47 41 91 33

CE

03/2009