BIO-RAD

HAEMOPHILUS TEST MEDIUM 63775

***ISOLATION MEDIUM FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF* HAEMOPHILUS INFLUENZAE**

IVD

1- INTENDED USE

H.T.M. (Haemophilus Test Medium) agar is a standardized solid medium recommended for antimicrobial susceptibility testing of *Haemophilus influenzae* (1, 2).

2- PRINCIPLE

H.T.M. medium consists of a Mueller-Hinton base supplemented with NAD and haemin, essential growth factors for *Haemophilus influenzae*.
The low thymine-thymidine concentration of this medium makes it perfectly adapted to test the susceptibility of *Haemophilus* to trimethoprim-sulphamethoxazole. It complies with CLSI M2-A7 standards (3).

3- HOW SUPPLIED

- Ready to use medium:
  - box of 10 Petri dishes (90 mm) (**HTM**) code 63775

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

Haemophilus Test Medium is prepared according to the formula described by Jorgensen et al. (1)

Mueller Hinton base	21
NAD	0.015
Haemin	0.015
Yeast extract	5
Agar	15
Final pH:	7.3 ± 0.1.

5- STORAGE

- Ready to use medium: at +2-8°C.

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

6- INSTRUCTIONS

Material:

- Material provided: Haemophilus Test Medium
- Specific material not provided:
  - Opacity standard equivalent to Mac Farland 0.5 standard.
  - Laboratory equipment necessary for antimicrobial susceptibility testing by the agar diffusion method.

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

Inoculation:

From a pure, fresh culture grown on chocolate agar, prepare a suspension in Mueller-Hinton broth or saline solution (0.9% NaCl) 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 agar diffusion method.
If the medium is stored at 4°C, allow it to adjust to room temperature (18-30°C).
If excessive moisture is observed on the surface, dry the dishes for 10 to 30 minutes in the incubator at 35°C before use.

- **Inoculation by flooding** (method recommended by the CA-SFM):

From the standardized inoculum, follow the procedure indicated by the CA-SFM concerning dilution of the initial suspension according to the bacterial species tested.
Flood the whole plate with the suspension obtained, then eliminate the excess suspension.

- **Inoculation by streaking** (Kirby-Bauer method recommended by the CLSI):

From the standardized inoculum, follow the CLSI guidelines to inoculate the dish:
- Immerse a sterile, non-toxic swab stick into the suspension.
- Remove surplus suspension by gently rotating the swab against the walls of the tube.
- Inoculate the plate with the swab to obtain a culture of confluent colonies.

Apply disks using a dispenser or forceps, pressing lightly.

Incubation:

Follow the current CLSI and CA-SFM guidelines.
- 18 to 24 hours at 35-37°C (CA-SFM)
- 16 to 18 hours at 35-37°C in an atmosphere containing 5% to 7% CO_2 (CLSI).

7- INTERPRETATION OF THE RESULTS

The interpretation of the antimicrobial susceptibility test using the disk diffusion method or the agar dilution method is described in periodic updates of the CA-SFM and CLSI guidelines.

8- PERFORMANCE/QUALITY CONTROL OF THE TEST

- Appearance of ready to use medium: clear, **amber-coloured** agar.
- The growth performances of Haemophilus Test Medium are verified with the following strains:

STRAINS	CULTURE RESULT AFTER 18 to 24 hours at 37°C in CO_2 atmosphere
Haemophilus influenzae CIP 52.151	Satisfactory growth
Haemophilus influenzae ATCC 49247	Satisfactory growth

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- LIMITS OF USE

- Pure, fresh cultures must always be used to obtain interpretable results.
- Some strains of *H. influenzae* requiring a higher than normal level of factor X (haemin) may show little or no growth on this medium.
- 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).

11- REFERENCES

1. Jorgensen, J.H., J.S. Redding, L.A. Maher, and A.W. Howell. 1987. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. J. Clin. Microbiol. 25:2105-2113.
2. Jorgensen, J.H., A.W. Howell, and L.A. Maher. 1990. Antimicrobial susceptibility testing of less commonly isolated *Haemophilus* species using *Haemophilus* test medium. J. Clin. Microbiol. 28:985-988.
3. National Committee for Clinical Laboratory Standards. CLSI. 2000. Approved standard: M2-A7. Performance standards for antimicrobial disks susceptibility tests, 4th ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.

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