



Reference : 01-706

Scharlau Microbiology - Technical data sheet

Product :
Haemophilus TEST AGAR BASE (HTM)

Also known as

HTM Agar, Haemophilus Test Medium Agar Base.

Specification

Solid medium for sensitivity testing of *Haemophilus influenzae* and *H. parainfluenzae* to antibiotics and other antimicrobials by the agar diffusion technique.

Formula * in g/L

Peptone.....	17.500
Solid meat infusion	2.000
Starch.....	1.500
Yeast extract.....	5.000
Agar.....	1.000

Final pH 7.3 ±0,1 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 21.5 g powder in 500 mL of distilled water. Heat gently until boiling and completely dissolved. Sterilize by autoclaving at 121 ° C for 15 minutes. Cool to 45-50 ° C and aseptically add the contents of a vial of Growth Supplement (HTM) for Haemophilus Ref.06-155LYO1. Homogenize the mixture and distribute aseptically in Petri dishes.

Description

Members of the genus *Haemophilus* are fastidious bacteria that require complex culture media for growth. The complexity of these media complicates the implementation of sensitivity tests and often causes antagonism between some nutrients and certain antimicrobial agents, which hinder both the reading and the reproducibility of results.

Scharlau Microbiology's HTM Agar Base has been formulated according to Jorgensen and colleagues that in the U.S., has been adopted by the Clinical and Laboratory Standards Institute (CLSI) and formally approved by the American National Standards Institute (ANSI) . The medium contains a low level of antimicrobial antagonists , so that sulphonamide testing can be carried out with a good degree of confidence. The transparency of this medium allows easy reading of inhibition zones through the bottom of the plate.

Technique

From *Haemophilus* discrete colonies, grown on chocolate agar for 20-24 hours, prepare a cell suspension in Mueller Hinton Broth (Ref. DC2136) or 1/4 Ringer's solution (Ref. DC6073) and adjust the cell concentration to McFarland 0.5, which corresponds to approximately 1-4 x 10⁸ CFU / mL. The preparation of the inoculum suspension must be careful because higher concentrations can cause false results with regard to β-lactam antibiotic resistance and if the concentration is below the specified growth is not confluent and it is difficult to read results. The cell suspension should be discarded if the inoculum is not used within 15 minutes after the adjustment of concentration.

The plates are inoculated by rubbing a swab moistened with the cell suspension, according to the Ericsson technique and left to dry for 3-5 minutes before placing antimicrobial discs on the surface, which should not be more than 4 per plate 100 mm in diameter.

The plates are incubated at 35 ± 2 ° C in a 5% CO₂ atmosphere for 18-20 hours before reading the inhibition zones.

For methodologies, refer to standard techniques referenced.

Necessary supplements

Growth Supplement (HTM) for *Haemophilus* (Art. No. 928240NL)

Vial contents:

Necessary amount for 500 mL of complete medium.

NAD..... 0,0075 g

Hemin..... 0,0075 g

Distilled water (Solvent)



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Quality control

Incubation temperature: 35°C ±2,0

Incubation time: 18-20h

Inoculum: Inoculate the entire agar surface and add antibiotic disks acc. to CLSI

Microorganism

Growth

Remarks

Haemophilus influenzae ATCC® 10211

Good

atmosphera 5%CO2

Haemophilus influenzae ATCC® 35056

Good

atmosphera 5%CO2

References

- BAUER, A.W., W.M.M. KIRBY, J.C. SHERRIS & M. TURCK (1966) Antibiotic Susceptibility Testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496
- ERICSSON, H.M. & J.C. SHERRIS (1971) Antibiotic Sensitivity Testing. Report of an internal collaborative study. Acta Pathol. Microbiol. Scand. 217(Suppl B):1-90
- JORGENSEN, J.H., J.S. REDDING, L.A. MAHER & A.W. HOWELL (1987). Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. J. Clin. Microbiol. 25:2105-2113
- CLSI (2006) Performance Standards for antimicrobial disk susceptibility Tests; Approved Standard - 9th Edition. Document M2-A9, Vol. 23, No. 1
- CLSI (2008) Performance Standards for Antimicrobial Susceptibility Testing; 8th Informational Supplement. Document M-100 S-18 Vol. 28, No. 1.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).