

HAEMOPHILUS TEST MEDIUM (HTM) BROTH

INTENDED USE

Remel Haemophilus Test Medium (HTM) Broth is a liquid medium recommended for use as a growth medium for *Haemophilus* spp. by Clinical and Laboratory Standards Institute (CLSI) procedures for the determination of minimal inhibitory concentrations (MIC).

SUMMARY AND EXPLANATION

The incidence of multidrug resistant clinical isolates of *Haemophilus influenzae* has increased in recent decades, mainly due to the spread of conjugative plasmids.¹ This development has effectively removed β -lactam antibiotics and cephalosporins as options for empirical treatment of infections in which *H. influenzae* is suspected. Broth media recommended in standard antimicrobial susceptibility methods are not adequate for growing fastidious organisms.² Complex growth-stimulating supplements, ordinarily used to grow *Haemophilus* spp., have been found to promote antagonism of certain antibiotics and result in poor reproducibility. In 1987 Jorgensen et al. described HTM Broth, an improved medium for antimicrobial susceptibility testing of *H. influenzae*.³ Doern et al. found HTM Broth to be superior to conventional broth media and allow for more reliable interpretation of growth endpoints.⁴ HTM Broth is transparent, stable, and can be used for testing trimethoprim and sulfonamide antimicrobial agents. Standard broth dilution methods using HTM Broth have been developed by CLSI for determining MIC concentrations of *H. influenzae* and *H. parainfluenzae*.⁵

PRINCIPLE

Beef extract and acid digest of casein supply amino acids, nitrogenous substances, vitamins, minerals, and other nutrients necessary for the growth of fastidious organisms. Yeast extract, hematin, and nicotinamide adenine dinucleotide (NAD) provide growth-stimulating factors without turbidity. Thymidine phosphorylase removes the antagonists to sulfonamides and trimethoprim, thereby improving endpoints. CLSI recommends 20-25 mg/l Ca++ (calcium) and 10-12.5 mg/l Mg++ (magnesium) for cation concentration.

REAGENTS (CLASSICAL FORMULA)*

Acid Digest of Casein.....	17.5 g	Hematin.....	15.0 mg
Yeast Extract.....	5.0 g	NAD.....	15.0 mg
Beef Extract.....	2.0 g	Mg++.....	10.0 mg
Starch.....	1.5 g	Thymidine Phosphorylase.....	200 IU
Ca++.....	20.0 mg	Demineralized Water.....	1000.0 ml

pH 7.3 \pm 0.1 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Implement appropriate procedures to verify presumptive identification of the test isolate as a *H. influenzae* or *H. parainfluenzae*.
2. Refer to the current version of CLSI M7 for appropriate testing protocol.²

INTERPRETATION OF THE TEST

1. MIC endpoints are determined by comparing the amount of growth in the wells (or tubes) containing antibiotic with the amount of growth in the growth-control well (or tube) which contains no antibiotic, for each set of tests. The growth-control well (or tube) must exhibit acceptable growth (≥ 2 mm button or definite turbidity) for the test to be considered valid.²
2. The MIC is defined as the lowest concentration of antimicrobial agent that completely inhibits growth of the test isolate as detected by the unaided eye.²
3. Refer to current versions of CLSI M7 and M100 for additional interpretation and reporting guidelines.^{2,5}

QUALITY CONTROL

All lot numbers of Haemophilus Test Medium (HTM) Broth have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.² Controls should be performed following established laboratory guidelines and in accordance with the recommendations of CLSI. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

**Haemophilus influenzae* ATCC® 49247
**Haemophilus influenzae* ATCC® 49766
***Haemophilus influenzae* ATCC® 10211

*CLSI recommended organism

**Recommended as a useful additional quality control strain to verify the growth-promotion properties of HTM.

INCUBATION

Ambient, 18-24 h @ 33-37°C
Ambient, 18-24 h @ 33-37°C
Ambient, 18-24 h @ 33-37°C

RESULTS

Good growth
Good growth
Good growth

LIMITATIONS

1. Organism suspensions adjusted incorrectly may result in inaccurate and misleading MIC interpretations.²
2. Improper storage of broth microdilution trays may result in loss of antibiotic potency and lead to false-resistant MIC interpretations.²
3. Contamination or other changes in the control strain may yield erroneous results.²

PERFORMANCE CHARACTERISTICS

A comparative study of MIC tests was performed on 197 isolates of *H. influenzae* (107 β -lactamase positive and 90 β -lactamase negative) using HTM Broth and Mueller Hinton Broth w/ Lysed Horse Blood (previous CLSI method). There was 100% correlation of mean MIC values between methods when tested with the following antibiotics: ampicillin, cefonicid, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, cefuroxime, and cephalothin.⁶

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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