remel

# HAEMOPHILUS TEST MEDIUM (HTM)

**INTENDED USE**

Remel Haemophilus Test Medium (HTM) is a solid medium recommended for use in qualitative procedures by the Clinical and Laboratory Standards Institute (CLSI) as the growth medium for antimicrobial disk diffusion tests with *Haemophilus* species.

**SUMMARY AND EXPLANATION**

The incidence of resistant strains of *Haemophilus influenzae* has increased in recent decades, underscoring the need for reliable susceptibility testing methods for this organism.[1] Modifications to standard susceptibility testing procedures have become necessary because of the fastidious nature of *H. influenzae*. Complex growth-stimulating supplements, ordinarily used to grow *Haemophilus* spp., have been found to promote antagonism of certain antibiotics and result in poor reproducibility.[2] In 1987, Jorgensen et al. described Haemophilus Test Medium (HTM),an improved medium for antimicrobial susceptibility testing of *H. influenzae.*[3] In their study, HTM compared favorably with the conventional medium, Mueller Hinton chocolate agar, is transparent, and allows more reliable interpretation of growth endpoints.

**PRINCIPLE**

HTM contains beef extract and acid hydrolysate of casein which provide amino acids, nitrogenous substances, vitamins, minerals, and other nutrients necessary for growth. Yeast extract, hematin, and nicotinamide adenine dinucleotide (NAD) provide growth-stimulating factors without the excessive turbidity that was inherent with the addition of animal blood products. Thymidine phosphorylase removes antagonists to sulfonamides and trimethoprim, thereby improving endpoints. Agar is the solidifying agent. Mueller Hinton base is accepted by both CLSI and the World Health Organization as a standardized, reproducible medium formulated to be low in antagonists to sulfonamides and trimethoprim.[4]

In the disk diffusion test, disks impregnated with a specific concentration of the desired antibiotic are placed on the surface of an inoculated HTM plate. The antibiotic diffuses into the agar creating a gradually changing gradient of the drug concentration around the disk. If the organism is susceptible, the microbial growth around the periphery of the disk is inhibited. If the organism is resistant, growth is not inhibited. The zones of inhibition are measured, correlated with minimum inhibitory concentration (MIC) values, and compared to CLSI interpretative criteria to determine the degree of susceptibility.

**REAGENTS (CLASSICAL FORMULA)***

| | | |
|---|---|---|
| Acid Digest of Casein | 17.5 | g |
| Yeast Extract | 5.0 | g |
| Beef Extract | 2.0 | g |
| Starch | 1.5 | g |
| Hematin | 15.0 | mg |
| NAD | 15.0 | mg |
| Thymidine Phosphorylase | 200.0 | IU |
| Agar | 17.0 | g |
| Demineralized Water | 1000.0 | ml |

pH 7.3 ± 0.1 @ 25°C

*Adjusted as required to meet performance standards.

**PRECAUTIONS**

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

**STORAGE**

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

**PRODUCT DETERIORATION**

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

**SPECIMEN COLLECTION, STORAGE, AND TRANSPORT**

Specimens should be collected and handled following recommended guidelines.[5]

**MATERIALS REQUIRED BUT NOT SUPPLIED**

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) McFarland 0.5 standard or equivalent (REF R20410), photometric device (optional), (7) Antimicrobial susceptibility disks, dispensing apparatus, (8) Sliding caliper, ruler, or template, (9) CLSI *Performance Standards for Antimicrobial Disk Susceptibility Tests*; Approved Standard (current edition).

**PROCEDURE**

1. Implement appropriate test methods to ensure presumptive identification of the test isolate as a *Haemophilus* species.
2. Inoculum should be prepared in Mueller Hinton broth or saline using the direct colony method.
   a. Prepare a direct suspension of the test isolate from an overnight culture on chocolate agar (preferably 20-24 hours).
   b. Adjust turbidity of the suspension visually to a McFarland 0.5 standard or equivalent, or use a photometric device. This suspension will contain approximately 1 to 4 x $10^8$ CFU/ml.
3. Inoculate agar plates within 15 minutes of preparing organism suspension.
4. Immerse a sterile swab into the suspension. Rotate the swab against the side of the tube above the fluid level to remove excess fluid.
5. Inoculate the dried surface of the plate in three planes by rotating the plate approximately 60 degrees each time.
6. The lid may be left ajar for 3 to 5 minutes, but no longer than 15 minutes, for the inoculum to be absorbed before applying antimicrobial susceptibility disks.
7. Apply disks individually or by using an antimicrobial disk dispenser. Do not place more than 9 antimicrobial disks per 150 mm plate or 4 disks per 100 mm plate. Disks should be no closer than 24 mm from center to center. Because some drugs diffuse almost instantaneously, do not relocate a disk once it has come in contact with the agar surface. Each disk must be pressed down to ensure complete contact with the agar surface.
8. Invert plates and place in the incubator within 15 minutes of disk application.
9. Incubate plates in 5% $CO_2$ at 35°C.
10. Plates should incubate a full 16-18 hours before measuring the zones of inhibition.

**INTERPRETATION**

1. Examine plate at 16-18 hours for a confluent lawn of growth and uniformly circular zones of inhibition around the antimicrobial disks. If individual colonies are present, the inoculum was too light and the test procedure must be repeated.
2. Invert the plate and position a few inches above a black, non-reflecting background illuminated with reflected light. Place the measuring device on the back of the inverted plate.
3. Use a sliding caliper or ruler to measure the diameters of the zones of complete inhibition (including the disk) to the nearest whole millimeter as judged by the unaided eye. A template prepared for this purpose may also be used.
4. Zone margins should be taken as the area showing no obvious growth than can be detected with the unaided eye. Faint growth or tiny colonies, which can only be detected by magnification at the edge of the zone of inhibited growth, should be disregarded.

5. With trimethoprim and the sulfonamides, antagonists in the medium may also allow slight growth. Therefore, with these drugs, slight growth (20% or less of the lawn of growth) should be disregarded and margins of heavy growth measured to determine zone diameters.
6. Interpret zones of inhibition by referring to the most current CLSI document and report as susceptible, intermediate, or resistant.4,6 Report the isolate susceptibility profile accordingly, paying special attention to all applicable footnotes.

**NOTES[4]:**

1. Rare β-lactamase negative, ampicillin-resistant (BLNAR) strains of *H. influenzae* should be resistant to amoxicillin-clavulanic acid, ampicillin-sulbactam, cefaclor, cefamandole, cefetamet, cefonicid, cefprozil, cefuroxime, loracarbef, and piperacillin-tazobactam, despite apparent *in vitro* susceptibility of some BLNAR strains to these agents.
2. Only results of testing with ampicillin, one of the third-generation cephalosporins, chloramphenicol, and meropenem should be reported routinely with all blood and cerebrospinal fluid isolates of *H. influenzae.*
3. Amoxicillin-clavulanic acid, azithromycin, cefaclor, cefprozil, clarithromycin, cefdinir, cefixime, cefpodoxime, cefuroxime axetil, loracarbef, and telithromycin are oral agents that may be used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not useful for management of individual patients. However, susceptibility testing of *Haemophilus* spp. with these compounds may be appropriate for surveillance or epidemiologic studies.
4. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. In most cases, a direct β-lactamase test can provide a rapid means of detecting ampicillin and amoxicillin resistance.

**QUALITY CONTROL**

All lot numbers of HTM have been tested using the following quality control organisms and have been found to be acceptable. This quality assurance testing conforms with or exceeds CLSI standards.[4,6] If aberrant quality control results are noted, patient results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|---|---|---|
| **Haemophilus influenzae* ATCC® 49247 | $CO_2$, 16-18h @ 35°C | Zone sizes within curren CLSI guidelines |
| **Haemophilus influenzae* ATCC® 49766 | $CO_2$, 16-18h @ 35°C | Good growth |
| ***Haemophilus influenzae* ATCC® 10211 | $CO_2$, 16-18h @ 35°C | Good growth |

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

**LIMITATIONS**

1. Improper storage of antimicrobial susceptibility disks may result in a loss of potency and false resistant interpretation.
2. Organism suspensions adjusted incorrectly may adversely affect results. Increased inoculum concentrations may lead to false-resistant results with some β-lactam antibiotics, particularly when β-lactamase producing strains of *H. influenzae* are tested.
3. Contamination or other changes in the control strain may yield erroneous results.
4. Failure to follow CLSI recommended procedures as described in the document may result in inaccurate results.

**PERFORMANCE CHARACTERISTICS**

In a comparative study of susceptibility tests with HTM and Mueller Hinton chocolate agar (previous CLSI method), 204 *Haemophilus* isolates were tested against 13 different antibiotics and the following test results were obtained.[7]

| | HTM | | |
|---|---|---|---|
| **MH Choc Agar** | **Sensitive** | **Intermediate** | **Resistant** |
| **Sensitive** | 1874 (83.5%) | 0 | 1 (0.4%) |
| **Intermediate** | 33 (1.5%) | 185 (8.2%) | 0 |
| **Resistant** | 11 (0.5%) | 11 (0.5%) | 129 (5.7%) |

Agreement: 97.5%

**BIBLIOGRAPHY**


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**PACKAGING**

Haemophilus Test Medium (HTM):
REF R04033, 15 X 150 mm Plates..... 10/Pk
REF R01503, 15 X 100 mm Plates..... 10/Pk

**Symbol Legend**

| | |
|---|---|
| **REF** | Catalog Number |
| **IVD** | In Vitro Diagnostic Medical Device |
| **LAB** | For Laboratory Use |
|  | Consult Instructions for Use (IFU) |
|  | Temperature Limitation (Storage Temp.) |
| **LOT** | Batch Code (Lot Number) |
|  | Use By (Expiration Date) |

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