



HAEMOPHILUS TEST MEDIUM

- For in vitro use only -

Catalogue No. PH15

Our Haemophilus Test Medium (HTM) is used for disk diffusion susceptibility testing with *Haemophilus* species.

HTM is an enriched Mueller Hinton Agar that contains casein peptone, yeast extract, and beef extract as nutritional sources. The addition of starch helps neutralize toxic substances in the medium as well as toxic metabolites produced during bacterial growth. Supplementation with NAD (V factor) and hematin (X factor) is necessary since they are essential growth factors required by *Haemophilus* species (except *H. aphrophilus*), for growth.

Our Haemophilus Test Medium was originally devised by Jorgenson et al. and conforms to recommendations put forth by the National Committee for Clinical Laboratory Standards (NCCLS). As with most Mueller Hinton mediums, the simplicity of HTM is advantageous for good batch-to-batch reproducibility with respect to disk susceptibility zone sizes; the transparent nature of the medium also makes zone interpretation easier and technically more accurate.

Formula per Litre of Medium

Beef Extract	2.0 g
Casein Peptone.....	17.5 g
Yeast Extract.....	5.0 g
Starch.....	1.5 g
Sodium Chloride	40.0 g
Agar.....	17.0 g
NAD	15.0 mg
Hematin	15.0 mg

pH 7.3 ± 0.1

Depth 4.0-mm ± 0.5-mm

Recommended Procedure

1. Allow medium to reach room temperature.
2. Prepare an inoculum of *Haemophilus* species in Mueller-Hinton Broth or 0.9% saline equal to 0.5 McFarland turbidity standard using colonies from an overnight chocolate plate.
3. Within 15 minutes of preparing the suspension, dip a sterile cotton swab into the prepared suspension and remove any excess inoculum by rotating the swab against the wall of the tube. Proceed to streak the surface of the medium in three directions at 60° intervals so that a heavy, confluent growth will be obtained.
4. If the surface is wet, allow the inoculum to dry and be absorbed by the medium by letting it stand at room temperature for 5 minutes.
5. Aseptically, apply antibiotic disks and tap them lightly to ensure complete contact is achieved with the surface of the medium. Ensure that the disks are situated suitably apart so that the susceptibility zones will not meet. If desired an automatic disk dispenser may also be used to apply the disks. No more than four antimicrobial disks should be placed on a single 100-mm plate and no more than nine antimicrobial disks should be placed on a single 150-mm plate, including not more than six of the following: third generation cephalosporins, aztreonam, imipenem or ciprofloxacin.
6. Within 15 minutes of disk application, invert and incubate plates in an aerobic environment supplemented with 5% CO₂ at 35°C.
7. Examine plates after 16-18 hours of incubation and measure the zones of inhibition around each disk.

Interpretation of Results

After the incubation period, a lawn of confluent growth should be observed with uniformly circular zones of inhibition around the antimicrobial disk. The zone margin should be taken as the area showing no obvious, visible growth. Measure the zones to the nearest whole millimeter while holding the plate a few inches from a black background and illuminated with reflected light. Disregard faint growth of tiny colonies which can be detected with difficulty at the edge of the zone of inhibition.

- *Strict adherence to testing protocols is required for accurate and reproducible results. Zone sizes may fluctuate due to inoculum size, rate of growth, length of incubation, and incubation environment*
- *If individual colonies are seen rather than a layer of confluent growth then the inoculum was too light and the organism must be tested again*
- *Our Haemophilus Test medium complies with the requirements of the National Committee for Clinical Laboratory Standards and is manufactured to contain low concentrations of thymine and thymidine*
- *Proper storage of antimicrobial disks is critical for accurate result, improper storage may cause a loss of potency and a falsely resistant result*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Zone Diameter (mm)					
	AMP 10-µg	C 30-µg	SXT 25-µg	MXF 30-µg	CEC 5-µg	CXM 30-µg
<i>Haemophilus influenzae</i> ATCC 49247	13 – 21	31 – 40	24 – 32	31 – 39		
<i>Haemophilus influenzae</i> ATCC 49766					25 – 31	28 – 36
<i>Haemophilus influenzae</i> ATCC 10211	Growth					

AMP = Ampicillin
MXF = Moxifloxacin
SXT = Trimeth/Sulfam

C = Chloramphenicol
CEC = Cefaclor
CXM = Cefuroxime

Storage and Shelf Life

Our Haemophilus Test Medium should be stored away from direct light at 4 to 8°C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PH15	Haemophilus Test Medium [Standard 15x100-mm plate]	10/pkg
PH15K	Haemophilus Test Medium [Kirby 15x150-mm plate]	5/pkg

References

1. Jorgensen JH et al. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. J Clin Micro 1987; 25:2105-13.

