HAEMOPHILUS TEST MEDIA (HTM)

PRODUCTS:

Plated Media:[a]

Haemophilus Test Medium (HTM)	P1692 (100mm), P3109 (150mm)
Mueller-Hinton Chocolate Agar	P2100, P3103

[a]see catalog for ordering options

INTENDED USE:

Haemophilus Test Media (HTM) is a solid media recommended for performing antimicrobial disk susceptibility testing of *Haemophilus* species. Mueller-Hinton Chocolate is used for the isolation and cultivation of fastidious organisms, particularly *Haemophilus* species.

PRINCIPLE:

The principle of disk diffusion susceptibility testing is based on the inhibition of microbial growth by antimicrobial agents on the surface of an inoculated agar plate. Antimicrobial agents diffuse into the agar from paper disks impregnated with a predetermined amount of an antibiotic. Rapidly growing microorganisms are considered susceptible or resistant if measured zones are within specified end points.

SUMMARY AND EXPLANATION:

Haemophilus Test Media is prepared using Mueller-Hinton Agar as the base. Mueller-Hinton Agar was originally recommended by Mueller and Hinton for the isolation of *Neisseria* species[10] and for detecting the resistance and responsiveness of gonococcal strains to sulfonamides.[3] Its original use has diminished and has since become the recommended substrate for disk susceptibility testing as described by Bauer et al.[1]

In 1987 Dr. Jorgenson et al.[5] developed new media for disk susceptibility testing of *Haemophilus influenzae*. Simply adding X and V factors (hemin and NAD) to Mueller-Hinton Agar allowed growth of some strains, however, many strains did not grow well enough with the addition of only these basic supplements to Mueller-Hinton base. To ensure adequate growth of *Haemophilus influenzae* strains for reliable susceptibility testing, yeast extract at a concentration of 0.5% was added to the media to provide an important growth-stimulating factor. Thymidine phosphorylase is not necessary for trimethoprim-sulfamethoxazole disk diffusion tests because yeast extract containing relatively little thymidine was chosen for the preparation of HTM. In a comparative study the HTM compared favorably with the conventional media, Mueller-Hinton Chocolate Agar (previous CLSI method).[2]

FORMULAS:

Approximate, per liter deionized filtered water.

(1) Haemophilus Test Media (HTM):

Beef Extract	2.0 g
Acid Hydrolysis of Casein	17.5
Starch	1.5
Yeast Extract	5.0
Agar	17.0
Bovine Hematin	15.0 mg
Nicotinamide Adenine Dinucleotide (NAD)	15.0

Final pH 7.3 ± 0.1 at 25°C

(2) Mueller-Hinton Chocolate Agar:

Beef Extract	2.0 g
Acid Hydrolysis of Casein	17.5
Starch	1.5
Agar	17.0
Hemoglobin	10.0
XV Factor Supplement	10.0 ml

Final pH 7.2 ± 0.2 at 25°C

PRECAUTIONS:*

For *in vitro* diagnostic use. Observe approved biohazard precautions. All infectious material should be steam sterilized in an autoclave at the required temperature and pressure. Consult an appropriate biosafety manual for further instructions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination or deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: Refer to the current Clinical and Laboratory Standards Institute (CLSI) documents titled, "Performance Standards for Antimicrobial Disk Susceptibility Tests."

Avoid extreme inoculum density. Never use undiluted overnight broth cultures for streaking plates. The use of a photometrically adjusted inoculum is highly recommended. Care must be exercised in preparing this suspension since a higher inoculum concentration may lead to false-resistant results with selected cephalosporin antimicrobials, particularly when tested with beta-lactamase producing strains of *H. influenzae*.

Disk susceptibility testing with HTM has been standardized only for rapidly growing strains of *Haemophilus*. Disk susceptibility testing on HTM is not adequate for isolates of *Haemophilus* that demonstrate a poor or slow growth rate on HTM after overnight incubation or that show marked strain-to-strain variation in growth rate.

Haemophilus influenzae producing beta-lactamase should be considered resistant to ampicillin and amoxicillin.

Unexplained QC results may occur as a result of a change in the QC organism's inherent susceptibility. Fresh cultures of the QC strains should be tested.

The disk diffusion method using HTM has been standardized only for *Haemophilus* spp. Other organisms should not be tested.

A nitrocefin ß-lactamase test should be performed on all clinically significant *Haemophilus* isolates.

In our HTM reproducibility study, PML was unable to consistently obtain the CLSI recommended quality control end points with the antibiotic azithromycin. If the end users quality control results for azithromycin are determined to be out of range, the results should not be reported.

PROCEDURES:

Specimen Collection: Not applicable since this media is not for primary isolation. This media is used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating this media. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.

Method of Use, Haemophilus Test Media (HTM): Prior to inoculation, the media should be brought to room temperature. Select 4-5 morphologically identical colonies taken directly from an overnight Chocolate Agar culture and transfer with a sterile needle or loop into a suitable broth, such as Mueller-Hinton. The suspension should be adjusted to a turbidity equivalent to a 0.5 McFarland Standard with a photometric device. Plated media should be inoculated within 15 minutes of making the suspension.

Dip a sterile swab into the broth suspension and then express the excess broth from it by rotating the swab several times against the tube wall above the broth. Inoculate an HTM plate by swabbing the inoculum on the surface of the plate three times, turning the plate 60 degrees between streaking.

After the inoculum has dried (3-5 minutes) with the plate lid closed, the predetermined antimicrobic disks are placed on the agar surface using aseptic technique. The disks should be pressed gently to assure complete contact with the agar surface. The disks should be at least 15 mm from the edge of the plate and far enough apart so that the zones of inhibition do not overlap. Nine disks can be dispensed on a 150 mm petri dish; no more than 4-5 disks may be used on a 100 mm dish.

Incubate inoculated plates at 35°C within minutes after application of the disks. Plates should be incubated in an atmosphere of 5-10% CO_2. Examine the plates after 16-18 hours and measure zone sizes to the nearest millimeter. Endpoints are determined as an inhibition of growth as seen visually by the use of refracted light. Ignore light, tiny growth that can be detected only by very close observation.

Interpretation: Refer to the current CLSI publication for specific zone diameters and interpretive zone criteria.[11]

Material Required but Not Supplied: Standard microbiological supplies and equipment such as those commonly found in a microbiological laboratory are not provided.

QUALITY CONTROL:*

The recommended CLSI quality control organisms used internally at PML to test Haemophilus Test Media, while simultaneously testing the viability of each disk and the methodology, are *H. influenzae*, ATCC #49247, and *H. influenzae,* ATCC #49766. The antibiotics used and the expected end points are those recommended in the CLSI publications mentioned in "Limitations" in this Technical Data Sheet. *H. influenzae*, ATCC #10211 may be used as a growth control. The following antibiotics are used to quality control each lot number of HTM: Cefotaxime, Ampicillin, Chloramphenicol, Trimeth/sulfa, Clarithromycin, Tetracycline, Ciprofloxacin, Cefaclor, Cefonicid, and Cefuroxime.

User Quality Control: The above quality control organisms are recommended by CLSI. Refer to CLSI document for detailed quality control parameters.[11] HTM should appear firm, clear, and straw in color. Mueller-Hinton Chocolate Agar should appear firm, opaque, and brown in color.

SPECIFIC PERFORMANCE CHARACTERISTICS:

Reproducibility Study: The performance of two production lots of PML HTM Agar were compared at two large medical centers in the northeast section of the United States using the CLSI recommended antimicrobial disk susceptibility test procedure M2-A5. A total of 12 antimicrobial agents (amoxicillin/clavulanic acid, ampicillin, azithromycin, cefaclor, cefixime, cefotaxime, cefuroxime, chloramphenicol, ciprofloxacin, imipenem, tetracycline, trimethoprim/sulfamethoxazide) were tested against 13 strains of *Haemophilus influenzae* in the study. Of the 13 strains, 3 were the American Type Culture Collection (ATCC®) strains recommended by CLSI as the organisms used to quality control test Haemophilus Test Agar. The remaining ten were clinical isolates: seven strains were ß-lactamase negative, while three strains produced ß-lactamase. The zone diameters obtained with each of the three lots of media were compared using the interpretive criteria from Table 2A in the M2-A5 document.

Within the two PML lot numbers, there were only six paired results where the zone sizes were in different interpretive categories. Of the six discrepancies, there were two with ampicillin, one with cefaclor, one with tetracycline, one with ciprofloxacin, and one with amoxicillin/clavulanic acid. In the case of ampicillin, the 1 to 2-mm difference that occurred would result in either an intermediate or resistant category (minor error). With cefaclor and tetracycline, a 2-mm difference from the mean zone diameters would have produced an interpretive category of either susceptible or intermediate (minor error). With ciprofloxacin and amoxicillin/clavulanic acid, antimicrobial agents with only one result category "susceptible," the 1-mm difference that occurred would produce an interpretive category of either susceptible or intermediate/resistant, also a minor error.

Quality Control Organisms Summary Data:

Antibiotic	Standard Deviation	Mean	Tests	Test Range	ATCC 49247 (CLSI Range)	ATCC 49766 (CLSI Range)
Amox/clav	1.5	19	20	(16-21)	(15-23)	
Ampicillin	1.29	19	20	(17-22)	(13-21)	
Azithromycin	2.24	21	20	(18-25)	(13-21)	
Azithromycin	1.99	21	30*	(18-25)	(13-21)	
Cefixime	1.39	28	20	(29-33)	(25-33)	
Cefaclor	1.25	28	20	(25-30)		(25-31)
Cefotaxime	1.99	33	20	(30-36)	(31-39)	
Cefuroxime	1.45	31	20	(29-34)		(28-36)
Chloramphenicol	1.54	34	20	(31-37)	(31-40)	
Ciprofloxacin	1.79	37	20	(34-40)	(34-42)	
Imipenem	1.62	26	20	(24-28)	(21-29)	
Tetracycline	0.72	18	20	(16-19)	(14-22)	
Trimeth/sulfa	1.53	28	20	(24-30)	(24-32)	

*To better determine the cause of the elevated standard deviation and the out-of-range results from the CLSI range for Azithromycin, a third study site performed 10 further tests on an additional lot number of HTM (See Azithromycin limitations).

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*For more detailed information, consult appropriate references.

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