BD™ Haemophilus Test Medium Agar (HTM)

INTENDED USE

BD Haemophilus Test Medium Agar (HTM) is used in the antimicrobial disc diffusion susceptibility procedure for clinical isolates of *Haemophilus* species as described in the Approved Standard M2, published by the Clinical and Laboratory Standards Institute (CLSI).[1]

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Mueller Hinton Agar supplemented with 1% hemoglobin and 1% IsoVitaleX Enrichment (Mueller Hinton Chocolate Agar) was the medium previously recommended for *Haemophilus influenzae*.[2] Extensive studies performed by Jorgensen and colleagues have led to the development of Haemophilus Test Medium Agar (HTM).[3,4] This medium is Mueller Hinton agar supplemented with X factor (hemin or hematin), V factor (nicotinamide adenine dinucleotide, NAD) and yeast extract.

A major advantage of HTM Agar compared with Mueller Hinton Chocolate Agar is optical clarity, permitting zone diameter measurements from the bottom of the dish as is the standard test procedure for nonfastidious organisms on Mueller Hinton Agar. Further, HTM Agar contains low levels of thymidine and is, therefore, suitable for testing trimethoprim/ sulfamethoxazole.

Interpretive criteria for the antimicrobial susceptibility testing of are provided in the CLSI document M100 (M2), which is included with CLSI document M2.[1] This document should be consulted for further details.

BD Haemophilus Test Medium Agar consists of Mueller Hinton Agar supplemented with X factor (hemin or hematin), V factor (nicotinamide adenine dinucleotide, NAD) and yeast extract.

REAGENTS

BD Haemophilus Test Medium Agar (HTM)

Formula* Per Liter Purified Water

Beef Extract	2.0 g
Acid Hydrolysate of Casein	17.5
Starch	1.5
Agar	17.0
Yeast Extract	5.0
Hematin	0.015
Nicotinamide Adenine Dinucleotide (NAD)	0.015

pH 7.3 ± 0.1

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

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration. Excessive shrinkage of this medium due to desiccation may lead to false susceptibility results.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

For user quality control, the CLSI [1] or, if applicable, national standards should be consulted. Principally, the procedures described below in **Test Procedure** should be performed, using the reference strains mentioned in **Materials Not Provided**.
BD Haemophilus Test Medium Agar (HTM) plates and the antimicrobial disks used should be tested at least twice weekly for proper performance.
Appearance of uninoculated medium: light amber.

PROCEDURE

Materials Provided

BD Haemophilus Test Medium Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

1. Tubed inoculum broth, such as **BD Trypticase™ Soy Broth** (Soybean-Casein Digest Broth) or Mueller Hinton II Broth (cation-adjusted), tubed in 5 ml volumes for preparation of a standard inoculum, and sterile broth or saline for dilution of inoculum.
2. Barium sulfate comparison standard (0.5 ml of 0.048 M $BaCl_2$ [1.175% w/v $BaCl_2.2H_2O$] to 99.5 ml of 0.18 M [0.36 N] H_2SO_4 [1% v/v]).
3. A photometric device for adjusting the turbidity of the inoculum suspension to be equivalent to the 0.5 McFarland standard.
4. As an alternative to the above materials (1-3), the **BD Prompt™ Inoculation System** (volumetric inoculum preparation device) can be used.[5,6]
5. Control cultures - *Haemophilus influenzae* ATCC™ 49247, ATCC 49766 and ATCC 10211.
6. Paper discs impregnated with specified amounts of antimicrobial agents, such as **BD Sensi-Disc™** susceptibility test discs.
7. Dispensing device, such as the Sensi-Disc 6-place dispenser.
8. Ruler or another device for measuring zone size in millimeters.
9. An incubator that produces an atmosphere containing 5% CO_2 or another device that produces a CO_2-enriched aerobic atmosphere.
10. A reagent for performing a rapid ß-lactamase test such as **BD Cefinase™** Discs.
11. Ancillary culture media, reagents, and laboratory equipment as required.

Specimen Types

This product is used for susceptibility testing of pure cultures of *Haemophilus* that have been isolated from clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

1. Prepare a Gram stain before starting susceptibility testing to confirm culture purity and to confirm tentative identification of *Haemophilus*.
2. Use several well-isolated colonies taken directly from an overnight (preferably 20 to 24 h) Chocolate Agar plate as the source of the inoculum.
3. A rapid ß-lactamase test should be utilized for rapid detection of strains that are resistant to penicillin, ampicillin or amoxicillin.
4. Prepare a suspension of the test organism in Mueller Hinton Broth, Mueller Hinton II Broth or 0.9% saline. This suspension should be adjusted to the turbidity of the 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1 - 4 x 10^8 CFU/ml. Care must be exercised in preparing this suspension because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antibiotics, particularly when beta-lactamase producing strains of *H. influenzae* are tested.[1]
5. Alternative methods of inoculum preparation involving devices that permit direct standardization of inocula without adjustment of turbidity, such as the **BD Prompt™**

Inoculation System, have been found to be acceptable for routine testing purposes including the testing of *H. influenzae*.[5,6]

6. Within 15 min of adjusting the turbidity of the inoculum, dip a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
7. Inoculate the entire agar surface of a **BD Haemophilus Test Medium Agar** plate three times, rotating the plate 60° between streakings to obtain even inoculation.
8. The lid may be left ajar for 3 to 5 min and the plate held at room temperature, but no longer than 15 min, to allow any surface moisture to be absorbed before applying the drug-impregnated discs.
9. Apply the discs by means of an antimicrobial disc dispenser, using aseptic precautions. Most antimicrobial agents produce larger zones of inhibition when tested against *Haemophilus* compared with other organisms. Therefore, no more than four antimicrobial discs should be placed on a single 100-mm plate, including not more than six of the following discs: third generation cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone, ceftizoxime), aztreonam, imipenem, or ciprofloxacin. After discs have been placed on the agar, tamp them with a sterile needle or forceps to make complete contact with the medium surface.
10. Within 15 min after the discs are applied, invert the plates and incubate for 16 to 18 h at 35°C in an aerobic atmosphere enriched with 5% carbon dioxide.
11. Streak a plate of **BD Haemophilus Test Medium Agar** with *H. influenzae* ATCC 10211 and incubate along with the susceptibility test plates to determine whether the medium supports adequate growth.

Results

1. Examine the plates after 16 to 18 h of incubation. A confluent "lawn" of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the test should be repeated.
2. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the plate, which is held over a black, non-reflecting background and illuminated from above.
3. The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected with difficulty near the edge of the obvious zone of inhibition.
   With trimethoprim and the sulfonamides, traces of antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20 or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

Calculation and Interpretation of Results

Refer to CLSI document M100 (M2) for interpretation of results obtained with clinical isolates of *Haemophilus*.[1] Results may be reported as resistant, intermediate or susceptible depending on the zone diameters obtained.

Organisms testing positive for beta-lactamase production should be considered resistant to ampicillin regardless of the zone diameters obtained. It should be noted that ampicillin-resistant strains of *H. influenzae* have been described which lack ß-lactamase activity.[7] Therefore, if the zone diameter indicates resistance to ampicillin, the isolate should be reported as resistant to that drug, even if the ß-lactamase test is negative.

Control cultures should be included each time a susceptibility test is performed or weekly if satisfactory performance can be documented according to the CLSI standard M2 which includes Tables (M100) with the correct zone diameters.[1]

Note: Informational supplements to CLSI Document M2, or revised versions, containing revised tables of antimicrobial discs and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. The complete standard and informational supplements can be ordered from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA. Telephone: ++1-610-688-1100. **www.clsi.org**

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

A. Reproducibility Study [8]

Three production lots of **BD Haemophilus Test Medium Agar** were evaluated using the antimicrobial disc susceptibility test procedure (M2-A4) recommended by the NCCLS at that time.[9] A total of 12 antimicrobial agents (amoxicillin/clavulanate, ampicillin, ampicillin/sulbactam, cefaclor, cefonicid, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, rifampin, tetracycline, trimethoprim/sulfametho-xazole) and 14 strains of *H. influenzae* were used in the study. Of the 14 strains, seven were American Type Culture Collection (ATCC) strains and the remaining seven were stock cultures, which included six clinical isolates and an industrial quality control culture. The zone diameters obtained with each of the three lots of media were compared with the interpretive criteria (Table 2A) in M2-A4.[9] In only six cases were zones obtained with one lot that would produce a different interpretive category compared with the other two lots. Of the six discrepancies, there were four with tetracycline, one with cefaclor and one with cefuroxime. In the case of tetracycline, the result would have produced an interpretive category of either susceptible or intermediate (minor error). With cefaclor and cefuroxime, the 1 mm difference that occurred would result in either an intermediate or resistant category, also a minor error.

In this study there were five strains of *H. influenzae* that produced beta-lactamase. With each of these strains, each HTM lot produced zone diameters with ampicillin that would be interpreted as being resistant. Two of the five strains also produced chloramphenicol acetyltransferase (CAT). The chloramphenicol zones obtained with these two strains would be consistent with chloramphenicol resistance. [10,11]

B. Comparison with Mueller Hinton Chocolate Agar [8]

The NCCLS disc procedure was performed with 213 clinical isolates of *H. influenzae* on HTM Agar and Mueller Hinton Chocolate Agar.[9,12] The antimicrobial agents tested were ampicillin, ampicillin/sulbactam, amoxicillin/clavulanate and chloramphenicol. These were the only antimicrobial agents for which zone diameter interpretive criteria had been defined for Mueller Hinton Chocolate Agar.[13,14] The following results were obtained.

		BD Haemophilus Test Medium Agar		
		S	**I**	**R**
Mueller Hinton	**S**	808	1	2
Chocolate Agar	**I**	0	0	0
	R	0	0	41

Agreement: 99.6% S = Susceptible I= Intermediate R= Resistant

There were two major errors and one minor error with ampicillin. In both major errors **BD Haemophilus Test Medium Agar** produced a resistant result, whereas Mueller Hinton Chocolate Agar produced a susceptible result. Both of these isolates were beta-lactamase positive. Therefore, the resistant result was correct. The one minor error was with an isolate that produced an intermediate result on HTM Agar and a susceptible result on Mueller Hinton Chocolate Agar.

C. Limitations of the Procedure

With some organism-antimicrobial agent combinations, the inhibition zone may not have a sharply demarcated edge, which could lead to incorrect interpretation.

Incorrect inoculum concentration may produce inaccurate results. Zones of inhibition may be too small if the inoculum is too heavy and they may be too large and difficult to measure if the inoculum is too light.

Improper storage of antimicrobial discs may cause a loss of potency and a falsely resistant result.

In vitro susceptibility of an organism to a specific antimicrobial agent does not necessarily mean that the agent will be effective in vivo. Consult appropriate texts for guidance in the interpretation of results.[2,15]

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Approved standard: M2. Performance standards for antimicrobial disk susceptibility tests. CLSI, Wayne, PA, USA.*Search for latest version at* **www.clsi.org**
2. Thornsberry, C., T.L. Gavan, E.H. Gerlach. 1977. Cumitech 6, New developments in antimicrobial agent susceptibility testing. Coordinating ed., J.C. Sherris. American Society for Microbiology, Washington, D.C
3. Jorgensen, J.H., J.S. Redding, L.A. Maher, and A.W. Howell. 1987. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. J. Clin. Microbiol. 25:2105-2113.
4. Jorgensen, J.H., A.W. Howell, and L.A. Maher. 1990. Antimicrobial susceptibility testing of less commonly isolated *Haemophilus* species using Haemophilus test medium. J. Clin. Microbiol. 28:985-988.
5. Baker, C.N., C. Thornsberry, and R.W. Hawkinson. 1983. Inoculum standardization in antimicrobial susceptibility testing: evaluation of overnight agar cultures and the rapid inoculum standardization system. J. Clin. Microbiol. 17:450-457.
6. Marsik, F., G. Evans, J. Fowler, and L. Thompson. 1989. Comparison of the BBL Prompt system, Abbott A-Just, and visual method for the preparation of *Haemophilus influenzae* inoculum for the Bauer-Kirby procedure, abstr. C-67, p. 404. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989.
7. Doern, G.V., J.H. Jorgensen, C. Thornsberry, D.A. Preston, T.A. Tubert, J.S. Redding, and L.A. Maher. 1988. A national collaborative study of the prevalence of antimicrobial resistance among clinical isolates of *Haemophilus influenzae*. Antimicrob. Agents Chemother. 32:180-185.
8. Data on file. BD Diagnostic Systems, Sparks, MD, USA.
9. National Committee for Clinical Laboratory Standards. 1990. Approved standard: M2-A4. Performance standards for antimicrobial disk susceptibility tests, 4th ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. Azemun, P., T. Stull, M. Roberts, and A.L. Smith. 1981. Rapid detection of chloramphenicol resistance in *Haemophilus influenzae*. Antimicrob. Agents Chemother. 20:168-170.
11. Doern, G.V., G.S. Daum, and T.A. Tubert. 1987. In vitro chloramphenicol susceptibility testing of *Haemophilus influenzae*: disk diffusion procedures and assays for chloramphenicol acetyltransferase. J. Clin. Microbiol. 25:1453-1455.
12. National Committee for Clinical Laboratory Standards. 1984. Approved standard: M2-A3. Performance standards for antimicrobial disk susceptibility tests, 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. National Committee for Clinical Laboratory Standards. 1987. Second Informational Supplement: M100-S2. Performance standards for antimicrobial susceptibility testing. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. Doern, G.V. and R.N. Jones. 1988. Antimicrobial susceptibility testing of *Haemophilus influenzae*, *Branhamella catarrhalis*, and *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. 32:1747-1753.
15. Woods, G.L., and J.A. Washington. 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods, p. 1327-1341. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C. USA.

PACKAGING/AVAILABILITY

BD Haemophilus Test Medium

Cat. No. 254058 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

Becton Dickinson GmbH
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

http://www.bd.com
http://www.bd.com/europe/regulatory/