Revisions

Rev from	Rev to	ECO #
0703	**0704**	**2942-04**

Notes:

1. BD Cat. Number 221966
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   Number of Pages: 2 Number of Sheets: 1
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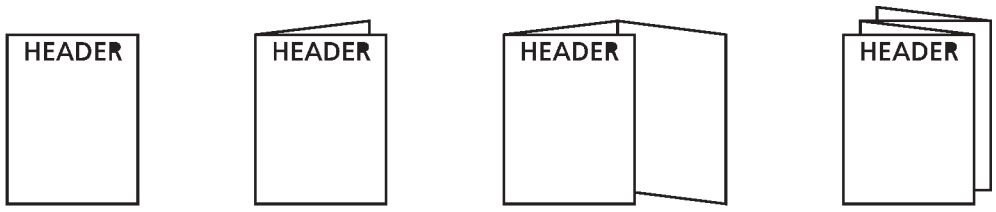
3. Style (see illustrations below): # 1



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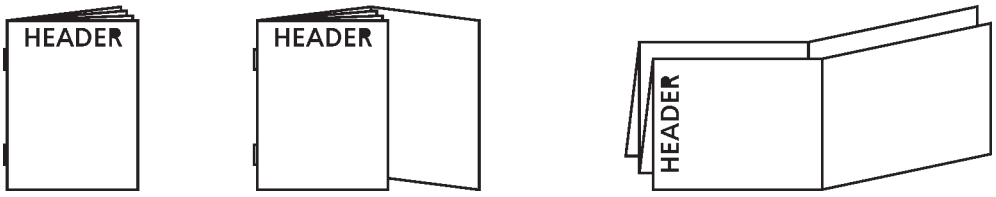

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4. See Specification Control Number N/A for Material Information
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	Category and Description Package Insert Haemophilus Test Medium Broth	Sheet: 1 of 3 Scale: 1 : 1	A

BD Haemophilus Test Medium Broth

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BBL™ Prepared Tubed Medium for Broth Dilution Susceptibility Testing of *Haemophilius influenzae*

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INTENDED USE

Haemophilus Test Medium Broth (HTM Broth) is intended for use in dilution antimicrobial susceptibility testing of *Haemophilus influenzae* as described in the Approved Standard M7, published by the National Committee for Clinical Laboratory Standards (NCCLS).[1]

SUMMARY AND EXPLANATION

The development of laboratory tests to determine the activity of antimicrobial agents has paralleled the development of these agents. Fleming used a serial dilution technique to measure the lowest concentration of penicillin that prevented growth of a test organism in broth.[2] Ericsson and Sherris have published an excellent review of the various methods for susceptibility testing and the relationship of dilution and diffusion methods.[3]

Broth dilution antimicrobial susceptibility tests (AST) are performed by inoculating serial dilutions (usually 2-fold) of the drug in a suitable liquid medium. The test may be performed in tubes (macrodilution), usually in 1.0 mL volumes, or in microtiter trays (microdilution) in volumes of 0.05 to 0.1 mL. Following incubation, the tubes or wells are examined for the presence of growth (turbidity or a pellet). The lowest concentration of an antimicrobial agent at which no visible growth occurs is defined as the minimal inhibitory concentration, or MIC.

The rationale for an MIC susceptibility test rather than the disc diffusion test is that it gives quantitiative information. It allows the clinician to correlate the amount of antimicrobial agent required to inhibit the growth of an organism *in vitro* and the achievable concentrations in the blood, urine, cerebrospinal fluid or bile. Effective antimicrobial therapy, however, also depends on other factors that must be considered.[4]

Broth dilution ASTs are usually performed in cation-adjusted Mueller Hinton Broth (CAMHB). However, this medium is not satisfactory for fastidious organisms such as *H. influenzae*.[1]

CAMHB supplemented with 2 to 5% laked horse blood and 10 µg/mL of nicotinamide adenine dinucleotide (NAD) was the medium previously recommended for susceptibility testing of *H. influenzae*.[5] Extensive studies performed by Jorgensen and colleagues have led to the development of Haemophilus Test Medium (HTM).[6] This medium is Mueller Hinton agar or broth supplemented with X factor (hemin or hematin), V factor (NAD) and yeast extract.

A major advantage of HTM Broth compared with Mueller Hinton Broth containing laked blood is optical clarity permitting easier interpretation of MIC endpoints. Further, HTM Broth ingredients are selected for low thymine and thymidine content and the medium is treated with thymidine phosphorylase to make it suitable for testing trimethoprim/sulfamethoxazole (SXT).

Interpretive criteria for the antimicrobial susceptibility testing of *H. influenzae* are provided in the NCCLS standard, M7.[1] This document and informational supplements should be consulted for further details.

PRINCIPLES OF THE PROCEDURE

The broth dilultion AST procedure is based on the serial 2-fold dilution in HTM Broth of antimicrobial agents which are then inoculated with the test culture to give a final concentration of $5x10^5$ CFU/mL. Following incubation at 35 ± 2°C for 20 to 24 h in an ambient air (non-CO_2) environment, the presence of growth (turbidity) of the organism is determined. The lowest concentration of antimicrobial agent showing no growth is the MIC of that organism for that agent.

The interpretation as to whether the organism is susceptible, resistant or intermediate in its response to the agent is made by comparing the MIC to those MIC interpretative standards in the NCCLS standard.[1]

Various factors have been identified as influencing broth dilution susceptibility tests. These include the medium, antimicrobial potency, inoculum concentration, pH, antimicrobial stability and β-lactamase production by test organisms.[1,3,7,8]

REAGENTS

Haemophilus Test Medium Broth

Approximate Formula* Per Liter Purified Water

Beef Extract	3.0	g
Acid Hydrolysate of Casein	17.5	g
Starch	1.5	g
Yeast Extract	5.0	g
Hematin	15.0	mg
Nicotinamide Adenine Dinucleotide	15.0	mg
Thymidine Phosphorylase	0.2	IU/mL

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

Warnings and Precautions:

For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 to 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labelled until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, precipitation, evaporation, or other signs of deterioration.

SPECIMEN COLLECTION AND HANDLING

This medium is not intended for direct use with clinical specimens or mixed cultures. The broth dilution susceptibility test is designed for use with pure cultures. A Gram stain and presumptive identification of *H. influenzae* are required.

PROCEDURE

Material Provided: Haemophilus Test Medium Broth

Materials Required But Not Provided:

1. Inoculum broth, such as cation-adjusted Mueller Hinton Broth tubed in 5-mL volumes or 0.9% saline, for preparation of a standardized inoculum.
2. A 0.5 McFarland barium sulfate standard for adjustment of inoculum (prepared by adding 0.5 mL of 0.048 M $BaCl_2$ [1.175% w/v $BaCl_2$ • 2 H_2O] to 99.5 mL of 0.18M [0.36 N] H_2SO_4 [1% v/v]).
3. A photometric device for verifying the turbidity of the barium sulfate standard.
4. Chocolate II Agar.
5. Control cultures - *Haemophilus influenzae* ATCC™ 49247 and ATCC 49766.
6. Antimicrobial agents and appropriate solvents (see ref. 1), or commercially prepared multiwell trays containing prediluted, dried or frozen, antimicrobial agents appropriate for testing *H. influenzae*.
7. A reagent or device for performing a rapid β-lactamase test, such as **BBL™ Cefinase™** Discs.
8. Ancillary culture media, reagents and laboratory equipment as required.

Test Procedure:

1. Prepare a Gram stain before starting susceptibility testing to confirm culture purity and tentative identification of *H. influenzae*.
2. A rapid β-lactamase test may be utilized to predict the activity of penicillin, ampicillin or amoxicillin. This rapid test provides valuable information earlier than tests requiring overnight incubation.
3. Prepare a suspension of the test organism in CAMHB. Use several well-isolated colonies taken directly from an overnight Chocolate Agar plate as the source of the inoculum. This suspension should be adjusted to the turbidity of the 0.5 McFarland standard (see Materials Required But Not Provided). This suspension will contain approximately 1×10^8 CFU/mL. Care must be exercised in preparing this suspension since higher inoculum concentration may lead to false-resistant results with certain cephalosporins, particularly with ß-lactamase-producing strains of *H. influenzae*.[1] Periodically perform dilutions and plate counts of inoculum suspensions to confirm that the inoculum contains approximately 1×10^8 CFU/mL.
4. The inoculum prepared above must be used within 15 min after preparation. It is then diluted in HTM Broth to achieve a final inoculum level in each tube or well of $5x10^5$ CFU/mL (not CFU per well).
5. Following inoculation, the tubes or trays are incubated for 20 to 24 h at 35°C in an aerobic atmosphere. To maintain the same incubation temperature for all cultures, microdilution trays are not to be stacked more than four high. To prevent drying out, the trays should be covered with plastic tape, a tight-fitting lid or placed in a plastic bag.[1]

User Quality Control:

1. Examine the medium for signs of deterioration as described under "Product Deterioration."

2. The control cultures, *Haemophilus influenzae* ATCC 49247 and 49766, should be included each time a susceptibility test is performed. Control limits are given in the NCCLS standard M7 or NCCLS informational supplement and indicate the correct performance of the entire procedure.[1]

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

RESULTS

1. Examine the tubes or trays after 20 to 24 h of incubation.
2. The MIC is the lowest concentration of antimicrobial that completely inhibits growth of the organism as detected by the unaided eye. Viewing devices that make it easier to read microdilution tests are available. The amount of growth in the wells or tubes containing the agent is compared with the amount of growth in the growth-control wells or tubes (no antimicrobial agent) used in each set of tests.
3. *H. influenzae* may be susceptible, intermediate or resistant for a given antimicrobial agent depending on the MIC value. Interpretive standards for MIC values with various drugs may be found in NCCLS standard M7[1] or may be obtained from the drug manufacturer. Organisms testing positive for β-lactamase production should be considered resistant to ampicillin regardless of the end point obtained.[1] It should be noted that ampicillin resistant strains of *H. influenzae* have been described which lack β-lactamase activity.[9] Therefore, if the end point indicates resistance to ampicillin, the isolate should be reported as resistant to that drug, even if the β-lactamase test is negative.

Note: Informational supplements to NCCLS Document M7, containing revised tables of antimicrobial agents and interpretive standards are published periodically. The latest tables should be consulted for current recommendations. For information on current publications, call BD Diagnostic Systems Technical Services at (800) 638-8663. The complete standard and informational supplements can be ordered from the National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. Telephone: (610) 688-1100.

Control cultures should be included each time a susceptibility test is performed or weekly if satisfactory performance can be documented according to the NCCLS standard.[1] The correct quality control MIC ranges will be found in that document.

LIMITATIONS OF THE PROCEDURE

With some organism-antimicrobial agent combinations, the end point may not be clear cut, which could lead to incorrect interpretation.

Incorrect inoculum concentration may produce inaccurate results. End points may be too low if the inoculum is too light. Inoculum concentrations higher than $5x10^5$ CFU/mL often lead to inappropriately high MIC's with certain cephalosporin antimicrobial agents, particularly when tested versus ß-lactamase-producing strains of *H. influenzae*.[1]

Improper storage of antimicrobials or antimicrobial-containing trays 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 references for guidance in the interpretation of results.[1,3,4,7,8]

PERFORMANCE CHARACTERISTICS

The NCCLS dilution susceptibility test procedure was performed with 241 (174 non-ß-lactamase-producers and 67 ß-lactamase-producers) clinical isolates of *H. influenzae* using HTM Broth and CAMHB with 2 to 5% laked horse blood and 10 µg/mL of NAD.[1,5] Twelve antimicrobials were tested. These were: trimethoprim/sulfamethoxazole, amoxicillin, ampicillin, ampicillin/sulbactam, cefaclor, cefonicid, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, imipenem, and rifampin. Of the 2,892 tests performed with HTM Broth, none were more than one 2-fold dilution greater or less than the results obtained with CAMHB with laked horse blood and NAD giving 100% correlation between the two media.

AVAILABILITY

Cat. No.	Description
221966	**BBL™** Haemophilus Test Medium Broth, Pkg. of 10 size K tubes

REFERENCES

1. National Committee for Clinical Laboratory Standards. 2003. Approved standard: M7-A6. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
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6. 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.
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