BD™ Mueller Hinton Agar with 5% Sheep Blood • BD Mueller Hinton Agar with 5% Sheep Blood (150 mm) • BD Mueller Hinton Agar with 5% Sheep blood, Square

INTENDED USE

BD Mueller Hinton Agar with 5% Sheep Blood, available in several plate formats, is used for disc diffusion susceptibility testing of clinical isolates of *Streptococcus pneumoniae* and other streptococci as standardized by the Clinical and Laboratory Standards Institute (CLSI).[1]

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

The Bauer-Kirby procedure is based on the diffusion through an agar gel of antimicrobial substances which are impregnated on paper discs.[2] In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated, and zones of inhibition around each disc are measured. The CLSI has written a performance standard for the Bauer-Kirby procedure and this document should be consulted for details.[1] Other national standards have been developed for antimicrobial susceptibility testing according to the Bauer-Kirby procedure. In those standards, the inoculum densities, the method of inoculation, the resulting zone sizes and the mode of interpretation may vary from the CLSI Standard. While common European standards for susceptibility testing do not exist, local national standards should be consulted if the CLSI Guidelines are not applicable.

Unsupplemented Mueller Hinton agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms such as *Streptococcus pneumoniae*. The CLSI Document M2 recommends Mueller Hinton Agar supplemented with 5% defibrinated sheep blood, and details both the quality control procedures and interpretive criteria for use with *S. pneumoniae* and other streptococci.[1]

REAGENTS

BD Mueller Hinton Agar with 5% Sheep Blood

Formula* Per Liter Purified Water

Beef Extract	2.0 g
Acid Hydrolysate of Casein	17.5
Starch	1.5
Agar	17.0 g
Sheep Blood, defibrinated	5%

pH 7.3 +/- 0.2

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

PRECAUTIONS

For In Vitro Diagnostic Use IVD. For professional use only. ②

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 appropriate CLSI [1] or, if applicable, national standards should be consulted. Principally, the procedures described below in **Test Procedure** should be followed, including the control strain, *S. pneumoniae* ATCC™ 49619, and the antimicrobial disks normally used by the laboratory should be tested at least twice weekly for proper performance.
The correct zone diameters will be found in Table 3A of CLSI Document M100 which is included with CLSI Document M2. [1]
Appearance of uninoculated medium: red (blood color), opaque.

PROCEDURE

Materials Provided

BD Mueller Hinton Agar with 5% Sheep Blood (provided in several different plate formats; see **Packaging/ Availability**). Microbiologically controlled.

Materials Not Provided

1. Inoculum broth in 5 ml amounts, such as Mueller Hinton II Broth (BBL Cat. No. 4397701, 16 x 102 mm tube) or 0.9% saline, for preparation of standard 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.[1,3]
5. Control culture - *Streptococcus pneumoniae* ATCC 49619.
6. Paper discs impregnated with specified amounts of antimicrobial agents, such as **BD Sensi-Disc™** susceptibility test discs.
7. Disc dispensing device, such as the **BD Sensi-Disc** Self-Tamping 6-, 8- or 12-Place Dispenser.
8. Device for measuring or interpreting zone diameters, such as callipers or a ruler.
9. An incubator that produces an atmosphere containing 5% CO_2, or another device that produces a similar CO_2-enriched atmosphere.
10. Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

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

Test Procedure

The direct colony suspension method should be used when testing *S. pneumoniae*. [1] Observe aseptic techniques.

1. Assure that a pure, fresh (=overnight) culture from a non-selective blood agar medium is available.
2. Suspend growth in broth such as Mueller Hinton II Broth or sterile 0.9% saline. Adjust the turbidity to be equivalent to the barium sulfate standard (0.5 McFarland standard). The turbidity of the standard and the test inoculum should be compared by holding both tubes in front of a white background with finely drawn black lines, or a photometric device can be used.

3. 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.[1,3]
4. 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.
5. Inoculate onto **BD Mueller Hinton Agar with 5% Sheep Blood** by streaking the entire agar surface of the plate three times, rotating the plate 60° between streakings to obtain even inoculation.
6. Replace the lid of the plate and hold the plate at room temperature for at least 3 min, but no longer than 15 min, to allow surface moisture to be absorbed before applying the drug-impregnated discs. Use no more than nine discs per 150 mm plate, or four discs per 90 and 100 mm plate. For penicillin testing of *S. pneumoniae*, use a 1 µg oxacillin disc.[4]
7. Incubate for 20 to 24 h at 35°C in an atmosphere of 5% CO_2.

Results

After incubation, confluent of growth should be visible. If only isolated colonies grow, the inoculum was too light and the test should be repeated.

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 sliding callipers, or a ruler, from top of the plate with lid removed. [5] 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 **BD Mueller Hinton Agar with 5% Sheep Blood**, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis.

Calculation and Interpretation of Results

Zone diameters should be compared with those in Table 2G for *S. pneumoniae* and 2H for other streptococci in the CLSI Document M100 (M2), which provide interpretive criteria. [1] Results obtained may then be reported as resistant, intermediate or susceptible. Observe special interpretive criteria for isolates of *S. pneumoniae* with oxacillin zone diameters of ≤ 19 mm mentioned in this standard (See also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

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

The disc diffusion susceptibility test is designed for use with pure cultures only. A Gram stain and a presumptive identification of the isolate are recommended before the susceptibility test is prepared.[1]

This medium is used for the susceptibility testing of *S. pneumoniae* and other streptococci against selected antimicrobials.[1,2,5] Note that for amoxycillin, ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, imipenem, and meropenem reliable disc diffusion test criteria for *S. pneumoniae* do not exist. The in vitro activity of these antimicrobials is best determined using an MIC method. [1]

For determination of penicillin susceptibility of *S. pneumoniae*, an oxacillin disc shall be used. Isolates of *S. pneumoniae* with oxacillin zone diameters of ≥ 20 mm are susceptible (MIC ≤ 0.06 mg/ml) to penicillin. Because zones of ≤ 19 mm with the oxacillin screening disc occur with penicillin-resistant, intermediate, and certain susceptible strains, a penicillin, meropenem, and cefotaxime or ceftriaxone MIC should be determined on all isolates of *S. pneumoniae* with oxacillin zones of ≤ 19 mm.[1]

For streptococci other than *S. pneumoniae*, the oxacillin test for determination of penicillin susceptibility is not recommended. For beta-hemolytic streptococci, use a penicillin or ampicillin disc. For *viridans* group streptococci, penicillin and oxacillin disc diffusion testing is not reliable; their susceptibility should be determined with MIC testing.[1]

Antimicrobial disc diffusion susceptibility testing using *Streptococcus pneumoniae* ATCC 49619, was performed in-house with cefaclor, cefprozil, chloramphenicol, erythromycin, ofloxacin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin. Following the test procedures described in M2-A5, twenty tests with the quality control strain and eight antimicrobic discs were performed over a period of 10 test days. For all eight antimicrobics, 100% (160/160) of the zone sizes fell within the expected zone size ranges published in M2-A5 and Table 3C of NCCLS Document M100-S6.[6,7] The standard deviation for tetracycline was less than 1 mm, and for all other antimicrobics, less than 2 mm.[8]
Reproducibility studies (3 times per day for 3 days) were done at two field sites with the antimicrobics listed above against *S. pneumoniae* ATCC 49619 and nine additional well-characterized *S. pneumoniae* strains. Zone diameter interpretive standards from Table 2C of NCCLS Document M2-A5 and Supplement M100-S6 were followed for each antimicrobic.[6,7]
Testing with chloramphenicol, erythromycin, ofloxacin, tetracycline, and vancomycin resulted in over 95% Category Agreement with the NCCLS reference method. Testing with trimethoprim/ sulfamethoxazole resulted in 90% Category Agreement with the NCCLS reference method. Reproducibility could not be determined for cefaclor and cefprozil due to the absence of interpretive standards for these two antimicrobics. Based on the studies outlined above, the use of cefaclor, ceprozil or trimethoprim/sulfamethoxazole is not recommended on this medium when testing *S. pneumoniae.* Additionally, references in the literature report excessive interpretive errors in disc diffusion testing with trimethoprim/sulfamethoxazole on Mueller Hinton sheep blood agar plates.[9]

With some organism-antimicrobial agent combinations, the inhibition zone may not have a sharply demarcated edge, which could lead to incorrect interpretation.
Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, agar depth, disc potency, inoculum concentration, age of inoculum, and pH.[2]
Incorrect inoculum concentration may produce incorrect 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 references for guidance in the interpretation of results.[2,10]
Do not use this medium for testing the susceptibility of bacteria other than *S. pneumoniae* and beta-hemolytic streptococci.

REFERENCES

1. Clinical and Laboratory Standards Institute. Approved Standard: M2. Performance standards for antimicrobial disk susceptibility tests. CLSI, Wayne, PA, USA.*Search for latest version at* **www.clsi.org**
2. Washington, J.A., and G.L. Woods. 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.
3. 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.
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6. National Committee for Clinical Laboratory Standards. 1993. Approved Standard: M2-A5. Performance standards for antimicrobial disk susceptibility tests, 5th ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
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10. Neumann, M.A., D.F. Sahm, C. Thornsberry, J.E. McGowan, Jr. 1991. Cumitech 6A, New developments in antimicrobial agent susceptibility testing: a practical guide. Coordinating ed., J.E. McGowan, Jr. American Society of Microbiology, Washington, D.C.

PACKAGING/AVAILABILITY

BD Mueller Hinton Agar with 5% Sheep Blood (90 mm **Stacker™** plates)

Cat. No. 254030	Ready-to-use Plated Media, cpu 20
Cat. No. 254080	Ready-to-use Plated Media, cpu 120

BD Mueller Hinton Agar with 5% Sheep Blood (150 mm)

Cat. No. 255080	Ready-to-use Plated Media, cpu 20

BD Mueller Hinton Agar with 5% Sheep blood, Square (120 x 120 mm)

Cat. No. 254517	Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

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