BD Sensi-Disc™ Susceptibility Test Discs

INTENDED USE

Sensi-Disc susceptibility test discs are used for semi-quantitative in vitro susceptibility testing by the agar disc diffusion test procedure of common, rapidly growing and certain fastidious bacterial pathogens. These include the *Enterobacteriaceae*, *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Enterococcus* spp., *Vibrio cholerae* and, by modified procedures, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and other streptococci.
Sensi-Disc susceptibility test discs loaded with bacitracin, oleandomycin, novobiocin, and polymyxin B are not used for determination of the susceptibility or resistance of isolates for therapeutic purpose, but are used for isolating and/or differentiating bacterial isolates. Sensi-Discs loaded with metronidazole have been used for screening isolates of strict anaerobes for metronidazole susceptibility by the broth disk dilution method. Consults the footnotes in Table 1.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Agar diffusion methods employing dried filter paper discs impregnated with specific concentrations of antimicrobial agents were developed in the 1940s. In order to eliminate or minimize variability in this testing, Bauer et al. developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium.[1,2]
Discs containing a wide variety of antimicrobial agents are applied to the surface of Mueller Hinton Agar plates (or Haemophilus Test Medium Agar for H. influenzae, GC II Agar with IsoVitaleX™ for N. gonorrhoeae or Mueller Hinton Agar with 5% Sheep Blood for S. pneumoniae, ß-hemolytic and viridans group streptococci) that have been inoculated with pure cultures of clinical isolates. Following incubation, the plates are examined and the zones of inhibition surrounding the discs are measured and compared with established zone size ranges for individual antimicrobial agents in order to determine the agent(s) most suitable for use in antimicrobial therapy.

Various regulatory agencies and standards-writing organizations published standardized reference procedures based on the Bauer-Kirby method. Among the earliest and most widely accepted of these standardized procedures were those published by the U.S. Food and Drug Administration (FDA)[3] and the World Health Organization (WHO).[4,5] The procedure was adopted as a consensus standard by the National Committee for Clinical Laboratory Standards (NCCLS) and is periodically updated.[6,7] The latest NCCLS documents should be consulted for current recommendations.

REAGENTS

Sensi-Disc brand discs are 6-mm discs prepared by impregnating high quality absorbent paper with accurately determined amounts of antibiotic or other chemotherapeutic agents. Discs are clearly marked on both sides with letters and numbers designating the agent and the drug content. (See chart giving concentrations of reactive ingredients.) The drug content of discs is assayed by the methods established by the FDA or by methods similar or comparable to those published in the United States Federal Register.[3]
Sensi-Disc agents are furnished in cartridges containing 50 discs each. The last disc in each cartridge is marked “X” and contains the drug as coded. Cartridges are for use in **BBL™ Sensi-Disc** Dispensers; these include a Single Disc Dispenser, an 8-Place Dispenser for 90 mm-style Petri dishes, 6- and 8-Place Self-Tamping Dispensers for 90 mm-style dishes and a Self-Tamping 12-Place Dispenser for 150 mm-style plates.

PRECAUTIONS

IVD . For professional use only.
Follow **PROCEDURES**; disc performance depends not only on disc potency, but on use of proper inoculum and control cultures, functional pretested plates, proper storage temperature and other factors.
Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.
Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

1. On receipt, store discs at -20 – +8°C. If the laboratory refrigerator is frequently opened and closed, and a suitable temperature is not maintained, place there a supply sufficient only for use within a week. Some discs (e.g., ß-lactams) should preferably be kept frozen at -20°C.
2. Allow containers to come to room temperature before opening. Return unused discs to the refrigerator when application of discs has been completed.
3. Use the oldest discs first.
4. Discard expired discs. Also, cartridges from which discs have been frequently removed during a week and discs left out overnight in the laboratory should be discarded, or else the discs should be tested for acceptable performance prior to continued use.
5. If the discs form incorrect zones with the recommended control organisms, the entire procedure should be checked; faulty zone size may be due to the disc, the inoculation, the preparation or depth of medium, or other factors.
The expiration date applies only to discs in intact containers, stored as directed. Discs from opened containers stored as indicated above can be used as long as the correct zone sizes with the appropriate control strains are met.

USER QUALITY CONTROL [6]

Antimicrobial disks should be tested at least twice weekly for proper performance.
E. coli ATCC™ 25922, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, *N. gonorrhoeae* ATCC 49226, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 35218 (ß -lactamase-producing strain), *E. faecalis* ATCC 29212 must be used to indicate the correct performance of the entire procedure. *E. faecalis* ATCC 29212 (or 33186) is also recommended for evaluating new lots of Mueller Hinton Agar for low thymine and thymidine content
See **PROCEDURE - Test Procedure** for preparation, inoculation, incubation, and reading.
Consult NCCLS Standard M2-A8 (M100-S15) or national standards for the expected zone sizes of the quality control strains.[6,7]

PROCEDURE

Material Provided

Sensi-Disc susceptibility test discs as labeled.

Materials Required But Not Provided

Ancillary culture media, reagents, quality control organisms and laboratory equipment required to perform disc diffusion susceptibility testing by the standardized procedure.
Prepare a 0.5 McFarland turbidity standard by adding 0.5 mL of 0.048 M $BaCl_2$ [1.175% (wt/vol) $BaCl_2 \times 2\ H_2O$] to 99.5 mL of 0.18 M [0.36N] H_2SO_4 [1% (vol/vol)]. Verify by using a spectrophotometer with a 1-cm light path and matched cuvette; absorbance at 625 nm should be 0.08 – 0.10.

Specimen Types
Specimens should not ordinarily be employed in this test. Instead, pure cultures must be used. See **PROCEDURE - Test Procedure**, which include preparation of inoculum. If possible, cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy.

Test Procedure
1. Preparation of inoculum with test and control cultures
    a. Perform a Gram stain. Use only pure cultures.
    b. Select three to five similar colonies and transfer with inoculation needle or loop into 4 – 5 mL of a suitable broth such as **Trypticase**™ Soy Broth (or Mueller Hinton Broth for fastidious organisms).
    c. Direct colony suspension method: Prepare a direct broth or saline suspension of colonies selected from an agar plate incubated overnight (a nonselective medium such as blood agar, or chocolate agar for *H. influenzae* and *N. gonorrhoeae*, should be used).
    d. Dilute, if required, to obtain turbidity equivalent to the 0.5 McFarland turbidity standard. For diluent, use sterile broth or saline. Alternatively, standardize the inoculum photometrically; to facilitate inoculum adjustment of rapidly growing organisms, the **Prompt™ Inoculation System** (volumetric inoculum preparation device) may be used.[8] Overnight broth cultures should not be used as inoculum.
2. Inoculation
    a. Within 15 min, dip a sterile cotton swab into the properly adjusted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid.
    b. Streak the entire agar surface of a Mueller Hinton Agar (or other appropriate agar) plate three times, turning the plate 60° between streakings to obtain even inoculation.
    c. The lid may be left ajar for 3 – 5 min, but no more than 15 min, to allow for any surface moisture to be absorbed before applying the drug-impregnated discs.
3. Select appropriate discs (such as recommended in reference 7, Tables 1 and 1A of M100-SI3 [M2]).
4. Apply the discs by means of a **BBL Sensi-Disc** dispenser, using aseptic precautions. Deposit discs so that the centers are at least 24 mm apart. It is preferable to deposit penicillin and cephalosporin discs so that they are no less than 10 mm from the edge of the Petri dish, and their centers are at least 30 mm apart. Avoid placing such discs adjacent to one another. With *H. influenzae, N. gonorrhoeae* and *S. pneumoniae*, use no more than nine discs per 150 mm plate or four discs per 90 mm plate. If discs have been placed on the agar with other than the Self-Tamping Dispensers, press them down with a sterile needle or forceps to make contact with the surface.
5. Within 15 min, place the plates agar side up in a 35°C incubator. *Haemophilus* spp., *N. gonorrhoeae*, *S. pneumoniae* and other streptococci should be incubated in an aerobic atmosphere enriched with 5% CO2.
6. Examine the plates after 16 – 18 h of incubation (20 – 24 h for *N. gonorrhoeae*, *S. pneumoniae* and other streptococci). A full 24 h of incubation is recommended for *Staphylococcus* spp. to detect methicillin/nafcillin/oxacillin-resistant staphylococci and *Enterococcus* spp. for vancomycin resistance. The diameters of the zones of complete inhibition are measured, as determined by gross visual inspection. Zones are measured to the nearest whole millimeter. For further details in measuring zones of inhibition, consult the reference.[6] If only isolated colonies grow, the inoculum is too light and the test should be repeated. Zones around discs containing different drugs are not comparable for the purpose of comparing activity of drugs.

Results [6,7]
Recommended interpretive criteria are based on usual dosage regimens and routes of administration in the U.S. Eventually, local standards should be consulted.
Compare recorded zone diameters with those given in the NCCLS Standard M2-A8 (M100-S15) or in national standards; results with a specific organism may be reported as Resistant, Intermediate or Susceptible. For some organism/antimicrobial combinations, the absence of resistant strains

precludes defining any results categories other than “Susceptible.” For strains yielding results suggestive of a “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed. If necessary, a dilution method usually will be the most appropriate testing method, which may require submitting the organism to a reference laboratory.[7]

The susceptibility of *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis can be reliably determined by the disc method, but may require extended incubation up to 24 h before reporting as susceptible.
Enterococci may be resistant to penicillin and ampicillin because of the production of low-affinity, penicillin-binding proteins (PBPs), or the production of ß-lactamase. The disc diffusion test can accurately detect isolates with altered PBPs, but it will not reliably detect ß-lactamase producing strains. The latter strains are best detected by using a direct ß-lactamase test;[6] e.g., with **Cefinase™** nitrocefin discs or chromogenic cephalosporin discs.
For Enterococcus spp., cephalosporins, aminoglycosides (except for high level resistance screening), clindamycin and trimethoprim/sulfamethoxazole may appear active in vitro but are not effective clinically and isolates should not be reported as susceptible.
Extended-spectrum ß-lactamases (ESBLs) are enzymes produced by gram-negative bacilli that arise by mutation in genes for common plasmid-mediated ß-lactamases. Strains of *Klebsiella* spp. and *E. coli* that produce ESBLs may be clinically resistant to therapy with penicillins, cephalosporins, or aztreonam, despite apparent in vitro susceptibility to some of these agents. Some of these strains will show zones of inhibition below the normal susceptible population but above the standard breakpoints for certain extended-spectrum cephalosporins or aztreonam; such strains should be screened for potential ESBL production by using the ESBL screening breakpoints before reporting results for penicillins, extended-spectrum cephalosporins or aztreonam. Other strains may test intermediate or resistant by standard breakpoints to one or more of these agents. In all strains with ESBLs the zone diameters for one or more of the extended-spectrum cephalosporins or aztreonam should increase in the presence of clavulanic acid as determined in phenotypic confirmatory testing. For all confirmed ESBL-producing strains, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. The decision to perform ESBL screening tests on all urine isolates should be made on an institutional basis, considering prevalence, therapy and infection control issues.[7]

For recognition of methicillin-resistant staphylococci, the oxacillin disc test is more likely to detect resistance than the use of methicillin or nafcillin discs. Therefore, the 1 µg oxacillin disc should be used to test for methicillin/oxacillin resistance. Any zone surrounding the oxacillin disc should be inspected carefully using transmitted light for small colonies or a light “film” of growth within the zone of inhibition after a full 24 h incubation. Methicillin-resistant staphylococci are often resistant to multiple classes of antimicrobial agents including aminoglycosides, macrolides, clindamycin, phenicols, quinolones, sulfonamides and tetracycline. The observation of multiple resistance should be a clue to the possibility of methicillin-resistance. However, strains of methicilin-resistant S. aureus that do not exhibit resistance to other classes of antimicrobial agents have been isolated from both inpatient and outpatient populations. If the disc diffusion test result is in doubt with a possible methicillin-resistant Staphylococcus spp., perform additional confirmatory tests as outlined in NCCLS document M7.[9] Methicillin/oxacillin-resistant S. aureus (MRSA) and coagulase negative staphylococci (MRS) should be reported as resistant (or not reported at all) to all penicillins, cephems, carbapenems and other ß-lactams, such as amoxicillin/clavulanic acid, ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam and imipenem, regardless of the in vitro test results with those agents. This is because most cases of documented infections due to methicillin-resistant staphylococci have responded poorly to ß-lactam therapy and convincing clinical data have yet to be presented that document clinical efficacy for those agents.[6] Isolates of staphylococci that are shown to carry the mecA gene, or the produce PBP 2a, the mecA gene product, should be reported as oxacillin resistant.
Interpretive criteria for coagulase-negative staphylococci correlate with the presence or absence of the gene encoding methicillin resistance (mecA) for *S. epidermidis*. These interpretive criteria may

overcall resistance for other coagulase-negative staphylococci, e.g., *S. lugdunensis* or *S. saprophyticus.* For serious infections with coagulase-negative staphylococci other than *S. epidermidis,* testing for mecA or the protein expressed by mecA, the penicillin binding protein 2a (PBP 2a, "also known as" PBP 2') may be appropriate for strains having zone diameters in the intermediate or resistant range. Isolates that are not shown to carry mecA or do not produce PBP 2a should be reported as oxacillin-susceptible.
It has been reported that disc susceptibility testing is not an accurate method for the determination of methicillin (oxacillin) susceptibility for coagulase-negative staphylococci (i.e., *S. saprophyticus*).[10] Routine testing of urine isolates of *S. saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections (e.g., nitrofurantoin, trimethoprim/sulfamethoxazole, or a fluoroquinolone).

A rapid ß-lactamase test (e.g., using **Cefinase** discs) may yield clinically relevant information earlier than results of a disc diffusion test with *Haemophilus* spp., *N. gonorrhoeae* and *Moraxella catarrhalis*; it is the only reliable test for detecting ß-lactamase-producing *Enterococcus* spp. A positive ß-lactamase test predicts resistance to penicillin, ampicillin and amoxicillin among *Haemophilus* spp., *N. gonorrhoeae* and *M. catarrhalis* and resistance to penicillin, including acylamino-, carboxy- and ureido-penicillins among staphylococci and enterococci. A negative ß-lactamase test does not rule out resistance due to other mechanisms. Do not test members of the *Enterobacteriaceae*, *Pseudomonas* spp. and other aerobic gram-negative bacilli because the results may not be predictive of susceptibility to the ß-lactams most often used for therapy. Accurate detection of ß-lactamase in staphylococci may require induction of the enzyme and incubation of a nitrocefin-based test for up to 1 h. Induction can be easily accomplished by testing the growth from the zone margin surrounding an oxacillin disc test. Care must be exercised to ensure accurate results, including testing of known positive and negative control strains at the time clinical isolates are examined.[6]
Susceptibility testing of penicillins and other ß-lactams approved by the U.S. Food and Drug Administration for treatment of Group A and B streptococci is not necessary for clinical purposes and need not be done routinely, since as with vancomycin, resistant strains have not been recognized. However, some strains of *S. agalactiae* may give penicillin-intermediate results.

Disc diffusion tests with ampicillin, penicillin, and rifampin for *Neisseria meningitidis* are unreliable. Minimal inhibitory concentration (MIC) tests should be used for these organisms.[7]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

The test as herein described applies primarily to rapidly growing aerobic pathogens. Fastidious bacteria, other than H. influenzae, *N. gonorrhoeae, S. pneumoniae* and other streptococci, should be tested by a dilution method.[9] Testing of anaerobes requires special procedures.[11]
For *Campylobacter, Corynebacterium* and *Bacillus* spp., data on the reliability of the agar diffusion test are not sufficient to recommend this method.
The classifications of Resistant, Intermediate and Susceptible vary only by one millimeter, which is within normal laboratory error. Some cultures may give a borderline zone that varies from day to day or from laboratory to laboratory; such cultures are relatively uncommon.
For detecting pneumococcal and enterococcal resistance, strictly adhere to NCCLS recommended methods.[6]

Antimicrobial agents other than those mentioned in the standards may be in current use. Susceptibility tests employing these agents should be interpreted on the basis of presence or absence of a definite zone of inhibition and should be considered as only qualitative until such time as interpretive zones have been established. All zone diameters should be recorded.
ESBL confirmatory testing is only valid when the four discs (cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid) are used simultaneously. Individual usage of these discs is not recommended by NCCLS.[6,7]

The method of inoculation, interpretation, and the limits of zone sizes given in the NCCLS standards may differ from national standards. [6, 12]

Sensi-Disc Vancomycin (254858) - **IMPORTANT NOTICE:** The ability to detect vancomycin-resistant *Staphylococcus aureus* (VRSA) with this product is unknown. Additional testing methods as recommended by the Centers for Disease Control and Prevention (CDC) should be used when performing susceptibility testing on *S. aureus* isolates, particularly methicillin-resistant *S. aureus* (MRSA). These tests include nonautomated MIC methods (e.g., broth microdilution or agar dilution) and a vancomycin agar screen test (Brain Heart Infusion Agar with 6 µg/ml of vancomycin). These methods require a full 24 hours of incubation to detect VRSA.
For additional information, please refer to the CDC website.

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PACKAGING/AVAILABILITY

BD Sensi-Discs

Packaged in glass tubes (with stoppers containing desiccant), 1 cartridge per tube. Sales unit: 10 tubes.

See Table 1 for availability of catalog numbers and products.

FURTHER INFORMATION

For further information please contact your local BD representative.

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Prompt is a trademark of 3M.
ATCC is a trademark of the American Type Culture Collection
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Table 1: **BD Sensi-Disc** products available in glass tubes

REF	Description
254744	AMIKACIN AN-10
254703	AMIKACIN AN-30
254741	AMOXICILLIN AMX-25
254718	AMOXYCILLIN + CLAVULANIC ACID AMC-30
254727	AMPICILLIN AM-10
254739	AMPICILLIN AM-25
254873	AZITHROMYCIN AZM 15
254749	AZLOCILLIN AZ-30
254750	BACITRACIN B-10 *
254755	CEFACLOR CEC-30
254758	CEFADROXYL CFR-30
254734	CEFAZOLIN CZ-30
254893	CEFEPIM FEP-30
254715	CEFOPERAZON CFP-30
254713	CEFOTAXIM CTX-30
254762	CEFOTIAM CFT-30
254711	CEFOXITIN FOX-30
254760	CEFSULODIN CFS-30
254878	CEFTAZIDIM CAZ-30
254722	CEFTRIAXON CRO-30
254775	CEFUROXIM CXM-30
254732	CEPHALEXIN CN-30
254704	CEPHALOTHIN CF-30
254725	CHLORAMPHENICOL C-30
254724	CIPROFLOXACIN CIP-5
254733	CLINDAMYCIN CC-10
254752	CLINDAMYCIN CC-2
254766	COLISTIN CL-10
254780	DOXYCYCLIN D-30
254731	ERYTHROMYCIN E-15
254786	FOSFOMYCIN + GLUCOSE-6-PHOSPHAT FF-120
254788	FOSFOMYCIN + GLUCOSE-6-PHOSPHAT FF-502
254785	FUSIDIC ACID FA-10
254726	GENTAMICIN GM-10
254797	IMIPENEM IPM-10
254799	KANAMYCIN K-30
254707	LINCOMYCIN L-15
254882	MEROPENEM MEM-10
254802	METRONIDAZOL MET-80**
254807	MEZLOCILLIN MZ-30
254730	NALIDIXIC ACID NA-30
254808	NEOMYCIN N-30
254710	NETILMYCIN NET-30
254702	NITROFURANTOIN FM-300

REF	Description
254855	NITROFURANTOIN + SULFADIAZIN U
254719	NORFLOXACIN NOR-10
254881	NOVOBIOCIN NB-5 *
254720	OFLOXACIN OFX-10
254819	OFLOXACIN OFX-5
254821	OLEANDOMYCIN OL-15 *
254822	OXACILLIN OX-1
254823	OXACILLIN OX-5
254824	PENICILLIN G P-0.8
254708	PENICILLIN G P-10
254700	PIPEMIDIC ACID PI-20
254712	PIPERACILLIN PIP-100
254832	PIPERACILLIN PIP-30
254828	POLYMYXIN B PB-300*
254709	SULFAMETHOXAZOL SMZ
254729	SULFAMETHOXAZOL + TRIMETHOPRIM SXT
254728	TETRACYCLIN TE-30
254815	TOBRAMYCIN NN-10
254816	TOBRAMYCIN NN-30
254714	TRIMETHOPRIM TMP-5
254844	TRIPLE SULFA SSS-0.25
254858	VANCOMYCIN VA-30

Footnotes to Table 1:
*These Sensi-Discs are used in a variety of isolation and identification procedures. They are not used for the susceptibility testing of isolates for therapeutic purpose.

254750 BACITRACIN B-10 and
254821 OLEANDOMYCIN OL-15:
These discs are used for the isolation of *Haemophilus influenzae* on nonselective media. After inoculation of the isolation plate, e.g., **BD Chocolate Agar (GC II Agar with IsoVitaleX)** or **BD Chocolate Agar (Blood Agar No. 2 Base)**, place a Bacitracin or Oleandomycin disc in the area of the first streak. After incubation, *Haemophilus influenzae* can be isolated from the area of the inhibition zone since it is resistant to Bacitracin and Oleandomycin, while most of the normal flora will be inhibited by these antimicrobials. [13-15]

254881 NOVOBIOCIN NB-5
This disc is used for the differentiation of staphylococci into novobiocin susceptible and resistant species: Perform agar diffusion test on Mueller Hinton II Agar and incubate 18 to 24 hours. Resistant: <16 mm; susceptible: ≥16 mm. *Staphylococcus saprophyticus, S. cohnii, S. xylosus* and a variety of other species are resistant, while *S. aureus, S. epidermidis, S. schleiferi*, and a variety of other species are sussceptible.[16]

254828 POLYMYXIN B PB-300
This disc is used for the differentiation of staphylococci into polymyxin susceptible and resistant species (same method as for novobiocin). Resistant <10 mm; susceptible ≥ 10 mm. *S. aureus, S. epidermidis, S. hyicus*, and *S. chromogenes* are resistant.[16] The disc is also used in a variety of other identification procedures.

254802 METRONIDAZOL MET-80
**This disk has been used for screening isolates of strict anaerobes (e.g., *Bacteroides* spp.) for metronidazole resistance by the broth disk elution method.[17,18] It must be noted that this method is no longer recommended by the NCCLS. [11] Also, strict anaerobes must not be tested by the disk diffusion method.