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AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19181, May 30, 1974, unless otherwise noted.

Subpart A—Susceptibility Discs

§ 460.1 Certification procedures for antibiotic susceptibility discs.

(a) *Standards of identity, strength, quality, and purity.* Antibiotic susceptibility discs are round flat discs that have a diameter of one-fourth inch and are made of clear absorbent paper containing antibiotic compounds. They are capable of absorbing moisture rapidly and the antibiotic is evenly distributed. The thickness is sufficient to assure rigidity and to have permitted the complete absorption of an adequate volume of antibiotic solution (approximately 0.02 milliliter). The identity of each disc is signified either by a color or by means of an identifying sign. The absorbent paper and dye or ink used must not affect either bacterial growth or the antibiotic. Each disc shall have a uniform potency that is equivalent to that contained in a standard disc prepared with one of the following quantities of antibiotic drugs:

- Ampicillin: 10 mcg.
- Bacitracin: 10 units.
- Carbenicillin: 50 mcg.
- Cefamandole: 30 mcg.
- Cefoxitin: 30 mcg.
- Cephalothin: 30 mcg.
- Chloramphenicol: 30 mcg.
- Clindamycin: 2 mcg.
- Colistin: 10 mcg.
- Erythromycin: 15 mcg.
- Gentamicin: 10 mcg.
- Kanamycin: 30 mcg.
- Methicillin: 5 mcg.
- Neomycin: 30 mcg.
- Novobiocin: 30 mcg.
- Oleandomycin: 15 mcg.
- Penicillin G: 10 units.
- Polymyxin B: 300 units.
- Rifampin: 5 mcg.
- Streptomycin: 10 mcg.
- Tetracycline: 30 mcg.
- Tobramycin: 10 mcg.
- Vancomycin: 30 mcg.

The standard discs used to determine the potency shall be made of paper as described in §460.6(d). Each antibiotic compound used to impregnate such standard discs shall be equilibrated in terms of the working standard designated by the Commissioner for use in determining the potency or purity of such antibiotic.

(b) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may contain a desiccant, and each may be packaged in combination with containers of suitable discs of drugs other than those described in paragraph (a) of this section. Such other discs shall be suitable only if the manufacturer and packer have submitted to the Commissioner information of the kind described in §431.17 of this chapter, and such information has been accepted by the Commissioner.

(c) *Labeling.* Each package of discs shall bear on its label or labeling, as hereinafter indicated, the following:

(1) On the outside wrapper or container and the immediate container:

(i) The batch mark.

(ii) The name and potency of each disc in the batch according to the following:

Name of disc	Content of antibiotic in micrograms or units per disc
Ampicillin-class disc	10 mcg. ampicillin.
Bacitracin disc	10 units bacitracin.
Carbenicillin disc	50 mcg. carbenicillin.
Cefamandole disc	30 mcg. cefamandole.
Cefoxitin disc	30 mcg. cefoxitin.
Cephalosporin-class disc	30 mcg. cephalothin.
Chloramphenicol disc	30 mcg. chloramphenicol.
Colistin disc	10 mcg. colistin.
Erythromycin disc	15 mcg. erythromycin.
Gentamicin disc	10 mcg. gentamicin.
Kanamycin disc	30 mcg. kanamycin.
Lincomycin-class disc	2 mcg. clindamycin.
Neomycin disc	30 mcg. neomycin.
Novobiocin disc	30 mcg. novobiocin.
Oleandomycin disc	15 mcg. oleandomycin.
Penicillin-class disc	10 units penicillin G.
Penicillinase-resistant penicillin-class disc	5 mcg. methicillin.
Polymyxin B disc	300 units polymyxin B.
Rifampin disc	5 mcg. rifampin.
Streptomycin-class disc	10 mcg. streptomycin.
Tetracycline-class disc	30 mcg. tetracycline.
Tobramycin disc	10 mcg. tobramycin.
Vancomycin disc	30 mcg. vancomycin.

(iii) The statement "Expiration date _____", the blank being filled in with the date that is 6 months after the month during which the batch was certified, except that the blank may be

filled in with a date that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such longer period of time. If it is a packaged combination of discs of two or more drugs, its outside wrapper shall bear only one expiration date, and that date shall be the date that is required for the shortest dated discs contained in the package.

(iv) The statement "For laboratory use only".

(2) On the circular or other labeling within or attached to the package, adequate directions for the use of such discs, including the following recommended method:

STANDARDIZED DISC SUSCEPTIBILITY TEST

DIRECTIONS FOR USE

Quantitative methods that require the measurement of zone sizes give the most precise estimates of antibiotic susceptibilities. The following outline describes such a procedure. Minor variations from this procedure may be used if the resulting procedure is standardized according to the results obtained in the laboratory from adequate studies with control cultures.

A. PREPARATION OF CULTURE MEDIUM AND PLATES

1. Melt previously prepared and sterilized Mueller-Hinton agar medium and cool to 45°-50° C.

2. For the purpose of testing certain fastidious organisms such as streptococci and *Haemophilus* species, 5 percent defibrinated human, horse, or sheep blood may be added to the above medium which is "chocolatized" when indicated.

3. To prepare the plates, pour the melted medium into Petri dishes on a level surface to a depth of 4 millimeters.

4. Let the medium harden and allow to stand long enough for excess moisture to evaporate. (For this purpose plates may be placed in an incubator at 35°-37° C. for 15-30 minutes or allowed to stand somewhat longer at room temperature.) There should be no moisture droplets on the surface of the medium or on the petri dish covers. The pH of the solidified medium should be 7.2-7.4. Satisfactory plates may be used immediately or refrigerated. Plates may be used as long

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as the surface is moist and there is no sign of deterioration.

NOTE: Commercially prepared agar plates meeting the above specifications may be used.

B. PREPARATION OF INOCULUM

1. Select four or five similar colonies.
2. Transfer these colonies (obtained by touching the top of each colony in turn with a wire loop) in turn to a test tube containing about 5 milliliters of a suitable liquid medium such as soybean-casein digest broth, U.S.P.
3. Incubate the tube at 35°-37° C. long enough (2 to 8 hours) to produce an organism suspension with moderate cloudiness. At that point the inoculum density of the suspension should be controlled by diluting it, or a portion of it, with sterile saline to obtain a turbidity equivalent to that of a freshly prepared turbidity standard obtained by adding 0.5 milliliter of 1.175 percent barium chloride dihydrate (BaCl₂·2H₂O) solution to 99.5 milliliters of 0.36 N (1.0 percent) sulfuric acid. Other suitable methods for standardizing inoculum density may be used; for example, a photometric method. In some cases it may be possible to get an adequate inoculum density in the tube even without incubation.

NOTE: Extremes in inoculum density should be avoided. Undiluted overnight broth culture should never be used for streaking plates.

C. INOCULATING THE PLATES

1. Dip a sterile cotton swab on a wooden applicator into the properly diluted inoculum. Remove excess inoculum from the swab by rotating it several times with firm pressure on the inside wall of the test tube above the fluid level.
2. Streak the swab over the entire sterile agar surface of a plate. Streaking successively in three different directions is recommended to obtain an even inoculum.
3. Replace the plate top and allow the inoculum to dry for 3 to 5 minutes.
4. Place the susceptibility discs on the inoculated agar surface and with sterile forceps, or needle tip flamed and cooled between each use, gently press down each disc to insure even contact. Space the discs even-

ly so that they are no closer than 10 to 15 millimeters to the edge of the petri dish and sufficiently separated from each other to avoid overlapping zones of inhibition. (Spacing may be accomplished by using a disc dispenser or by putting the plate over a pattern to guide the placement of discs.) Within 30 minutes, place the plate in an incubator under aerobic conditions at a constant temperature in the range of 35°-37° C.

5. Read the plate after overnight incubation or, if rapid results are desired, the diameters of the zone of inhibition may be readable after 6 to 8 hours incubation. In the latter case, the results should be confirmed by also reading the results after overnight incubation.

NOTE: Microbial growth on the plate should be just or almost confluent. If only isolated colonies are present the inoculum was too light and the test should be repeated.

Modifications of the inoculation procedure described in 1-3 above, such as the use of the agar overlay method described in Barry, A. L., Garcia, F., and Thrupp, L. D.: "An Improved Single-disk Method for Testing the Antibiotic Susceptibility of Rapidly-growing Pathogens." Amer. J. Clin. Pathol. 53:149-58, 1970,* a copy of which is on file with the Office of the Federal Register, may be used if the procedure is standardized to produce results with the control cultures that are equivalent to those obtained with the recommended cotton swab streak method.

D. READING THE PLATES

Measure and record the diameter of each zone (including the diameter of the disc) to the closest millimeter, reading to the point of complete inhibition as judged by the unaided eye. Preferably, read from the underside of the plate without removing the cover, using a ruler, calipers, transparent plastic gage, or other device. A mechanical zone reader may be used. If blood agar is used, measure the zones from the surface with the cover removed from the plate.

E. INTERPRETATION OF ZONE SIZES

Interpret the susceptibility according to the following table:

Antibiotic	Disc content	Diameter (millimeters) of zone of inhibition		
		Resistant	Intermediate	Susceptible
Ampicillin ¹ when testing gram-negative microorganisms and enterococci.	10 mcg	11 or less	12-13	14 or more.
Ampicillin ¹ when testing staphylococci and penicillin G—susceptible micro-organisms.	10 mcg	20 or less	21-28	29 or more.
Ampicillin ¹ when testing <i>Haemophilus</i> species	10 mcg	19 or less	20 or more.

*Copies may be obtained from: J. B. Lippincott Company, Attn: Circulation Man-

ager, East Washington Square, Philadelphia, PA 19105.

Antibiotic	Disc content	Diameter (millimeters) of zone of inhibition		
		Resistant	Intermedi-ate	Susceptible
Bacitracin	10 units	8 or less	9-12	13 or more.
Carbenicillin when testing <i>Proteus</i> species and <i>Escherichia coli</i>	50 mcg	17 or less	18-22	23 or more.
Carbenicillin when testing <i>Pseudomonas aeruginosa</i>	50 mcg	12 or less	13-14	15 or more.
Cefamandole ¹²	30mcg	14 or less	16-17	18 or more.
Cefoxitin ¹¹	30 mcg	14 or less	15-17	18 or more. ¹¹
Cephalothin when reporting susceptibility to cephalothin, cephaloridine, and cephalexin.	30 mcg	14 or less	15-17	18 or more. ²
Cephalothin when reporting susceptibility to cephaloglycin	30 mcg	14 or less	15 or more.
Chloramphenicol	30 mcg	12 or less	13-17	18 or more.
Clindamycin ³ when reporting susceptibility to clindamycin	2 mcg	14 or less	15-16	17 or more.
Clindamycin when reporting susceptibility to lincomycin	2 mcg	16 or less	17-20	21 or more.
Colistin	10 mcg	8 or less	9-10	11 or more.
Erythromycin	15 mcg	13 or less	14-17	18 or more.
Gentamicin	10 mcg	12 or less	13 or more.
Kanamycin	30 mcg	13 or less	14-17	18 or more.
Methicillin ⁵	5 mcg	9 or less	10-13	14 or more.
Neomycin	30 mcg	12 or less	13-16	17 or more.
Novobiocin	30 mcg	17 or less	18-21	22 or more. ⁶
Oleandomycin ⁷	15 mcg	11 or less	12-16	17 or more.
Penicillin G when testing staphylococci ⁸	10 units	20 or less	21-28	29 or more.
Penicillin G when testing other micro-organisms ⁸	10 units	11 or less	⁹ 12-21	22 or more.
Polymyxin B	300 units	8 or less	9-11	12 or more. ⁴
Rifampin when testing <i>Neisseria meningitidis</i> susceptibility only	5 mcg	24 or less	25 or more.
Streptomycin	10 mcg	11 or less	12-14	15 or more.
Tetracycline ¹⁰	30 mcg	14 or less	15-18	19 or more.
Tobramycin	10 mcg	11 or less	12-13	14 or more.
Vancomycin	30 mcg	9 or less	10-11	12 or more.

¹ The ampicillin disc is used for testing susceptibility to both ampicillin and hetacillin.
² Staphylococci exhibiting resistance to the penicillinase-resistant penicillin class discs should be reported as resistant to cephalosporin class antibiotics. The 30 mcg. cephalothin disc cannot be relied upon to detect resistance of methicillin-resistant staphylococci to cephalosporin class antibiotics.
³ The clindamycin disc is used for testing susceptibility to both clindamycin and lincomycin.
⁴ Colistin and polymyxin B diffuse poorly in agar and the accuracy of the diffusion method is thus less than with other antibiotics. Resistance is always significant but when treatment of systemic infections due to susceptible strains is considered, it is wise to confirm the results of a diffusion test with a dilution method.
⁵ The methicillin disc is used for testing susceptibility to all penicillinase-resistant penicillins; that is, methicillin, cloxacillin, dicloxacillin, oxacillin, and nafcillin.
⁶ Not applicable to medium that contains blood.
⁷ The oleandomycin disc is used for testing susceptibility to oleandomycin and troleandomycin.
⁸ The penicillin G disc is used for testing susceptibility to all penicillinase-susceptible penicillins except ampicillin and carbenicillin; that is, penicillin G, phenoxymethyl penicillin, and phenethicillin.
⁹ This category includes some organisms such as enterococci and gram-negative bacilli that may cause systemic infections treatable with high doses of penicillin G. Such organisms should only be reported susceptible to penicillin G and not to phenoxymethyl penicillin or phenethicillin.
¹⁰ The tetracycline disc is used for testing susceptibility to all tetracyclines; that is, chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, rolitetracycline, minocycline, and tetracycline.
¹¹ The cefoxitin disc should not be used for testing susceptibility of other cephalosporins.
¹² The cefamandole disc should not be used for testing susceptibility of other cephalosporins.

F. REFERENCE ORGANISMS

- Maintain stock cultures of *Staphylococcus aureus* (ATCC 25923)¹ and *Escherichia coli* (ATCC 25922).¹
- Test these reference organisms daily by the above procedure using antibiotic discs

representative of those to be used in the testing of clinical isolates.

- The individual values of zone sizes for the control organisms can be expected to fall in the ranges indicated in the following table:

Antibiotic	Disc content	Individual tests		
		Zone diameter in millimeters		Permitted millimeter difference ATCC 25923- ATCC 25922
		With <i>S. aureus</i> ATCC 25923 ¹	With <i>E. coli</i> ATCC 25922 ¹	
Ampicillin	10 mcg	24-35	15-20	7-17
Bacitracin	10 units	17-22
Cefamandole	30 mcg	28-34	24-31	-1-6.8
Cefoxitin	30 mcg	24-32	25-30	3-4
Cephalothin	30 mcg	25-37	18-23	5-16

Antibiotic	Disc content	Individual tests		
		Zone diameter in millimeters		Permitted millimeter difference ATCC 25923- ATCC 25922
		With <i>S. aureus</i> ATCC 25923 ¹	With <i>E. coli</i> ATCC 25922 ¹	
Chloramphenicol	30 mcg	19-26	21-27	-4-1
Clindamycin	2 mcg	23-29		
Colistin	10 mcg		11-15	
Erythromycin	15 mcg	22-30	8-14	10-19
Gentamicin	10 mcg	19-27	19-26	-2-3
Kanamycin	30 mcg	19-26	17-25	-1-4
Methicillin	5 mcg	17-22		
Neomycin	30 mcg	18-26	17-23	0-3
Novobiocin	30 mcg	22-31		
Oleandomycin	15 mcg	19-28		
Penicillin G	10 units	26-37		
Polymyxin B	300 units	7-13	12-16	-7--2
Streptomycin	10 mcg	14-22	12-20	-1-5
Tobramycin	10 mcg	19-29	18-26	
Tetracycline	30 mcg	19-28	18-25	0-6
Vancomycin	30 mcg	15-19		

¹ Available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

G. LIMITATIONS OF THE METHOD

The method of interpretation described in E above applies to rapidly growing pathogens and should not be applied to slowly growing organisms. The latter show larger zones of inhibition than those given in the table. Susceptibility of gonococci to penicillin, and of slow-growing strains, e.g., *Bacteroides* species and fastidious anaerobes to any antibiotic, should be determined by the broth-dilution or agar-dilution method unless specifically standardized diffusion tests are used.

(d) *Requests for certification; samples.*

(1) In addition to complying with the requirements of §431.1 of this chapter, a person who requests certification of a batch of antibiotic susceptibility discs shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, and, unless it was previously submitted, the date on which the latest assay of the antibiotic used in making such batch was completed, the potency of each disc, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(2) Such person shall submit in connection with his request results of the tests and assays made by him on an accurately representative sample of the batch for potency.

(3) Such person shall submit in connection with his request an accurately representative sample of the batch consisting of one disc for each 5,000 discs in the batch, but in no case less than 36 discs collected by taking single discs at intervals throughout the entire time of packaging the batch so that the quantities packaged during the intervals are approximately equal.

[39 FR 19181, May 30, 1974, as amended at 41 FR 7093, Feb. 17, 1976; 41 FR 35061, Aug. 19, 1976; 44 FR 10376, Feb. 20, 1979; 44 FR 20666, Apr. 6, 1979]

§460.6 Tests and methods of assay for potency of antibiotic susceptibility discs.

(a) *Culture media.* Use ingredients that conform to the standards prescribed by the United States Pharmacopeia or The National Formulary. In lieu of preparing the media from the individual ingredients, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the specified individual ingredients is permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(1) *Medium A:*

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.