

Rules and Regulations

Title 21—FOOD AND DRUGS

Chapter I—Food and Drug Administration, Department of Health, Education, and Welfare

SUBCHAPTER C—DRUGS

[DESI 90235]

PART 144—ANTIBIOTIC DRUGS; EXEMPTIONS FROM LABELING AND CERTIFICATION REQUIREMENTS

PART 147—ANTIBIOTICS INTENDED FOR USE IN THE LABORATORY DIAGNOSIS OF DISEASE

Antibiotic Susceptibility Discs

In a notice published in the *FEDERAL REGISTER* of April 10, 1971 (36 F.R. 6899), the Commissioner of Food and Drugs proposed amendments to Part 147 (21 CFR 147) regarding antibiotic susceptibility discs. Interested persons were invited to submit comments in response to the notice of proposed rule making within 30 days; this time period was extended to June 9, 1971, by a notice published May 25, 1971 (36 F.R. 9446).

The proposal was based on conclusions made by the Commissioner of Food and Drugs after having considered reports and hoc Advisory Committee on Antibiotic Drugs; reports from the National Academy of Sciences-National Research Council, Drug Efficacy Study Group; and other relevant material. The notice proposed to amend the antibiotic drug regulations to provide for a standardized antibiotic disc susceptibility test; to delete provisions regarding carbomycin, dihydrostreptomycin, nystatin, ristocetin, and viomycin discs; and to delete provisions regarding certain other antibiotic discs that represent analogues and the discs containing an antibiotic, a dehydrated medium, and triphenyltetrazolium chloride.

Comments were received from the American Society for Microbiology, Center for Disease Control, College of American Pathologists, National Committee for Clinical Laboratory Standards, Pharmaceutical Manufacturers Association, nine interested manufacturers, and four universities. All comments were carefully considered. There is general agreement on the validity of single disc susceptibility testing and the need for standardized testing procedures. Most of those commenting agreed in general on the standardized method for performing antibiotic disc susceptibility tests as described in the proposal. The comments and the Commissioner's conclusions based on his evaluation of them may be summarized as follows:

1. A number of comments were received concerning the appropriateness of

methicillin discs for testing susceptibility to the penicillinase-resistant family of penicillins. The Commissioner, after consideration of relevant information, concludes that methicillin is an appropriate representative of the family of penicillinase-resistant penicillins.

2. Requests were received to provide for one or more additional analogous members of certain classes of antibiotics. However, such requests for additional analogues were not supported by substantial clinical and laboratory information to show a unique advantage for a disc of any such additional analogue. Therefore, the Commissioner concludes that at this time there is a lack of data to support the additional analogues requested.

3. Several comments questioned the use of only one standardized disc testing method and recommendations were made for alternate disc testing methods. The Commissioner, having considered the advisability of recommending more than one standardized disc testing method, concludes that because the proposed standardized antibiotic disc susceptibility test offers such advantages in providing dependable testing results to physicians at the present time it is not in the best interest of good medical care to recommend alternate disc methods that have not been established to be as reliable.

4. Comments were received that susceptibility discs other than those containing antibiotic drugs should be certified in some manner by the Food and Drug Administration. The Commissioner recognizes the need for dependable results with all discs used in determining the susceptibility of pathogenic microorganisms to drugs; however, the proposal is limited to those drugs, specifically antibiotics, which are subject to the certification provisions of the act (21 U.S.C. 357). FDA published in the *FEDERAL REGISTER* on August 17, 1972 (37 F.R. 16613), a proposal establishing a procedure directed toward assuring adequate clinical laboratory results for all diagnostic products.

5. Several comments concerned the authority under the Food, Drug, and Cosmetic Act to limit antibiotic susceptibility discs to only those deemed representative of the class. There was also concern that limitation to one disc, representative of a family of antibiotics, would result in a tendency to select for treatment antibiotic dosage forms which contain the same antibiotic as contained in the disc. The policy of using a representative drug of each antibiotic class is not intended to restrict therapy to only the specific antibiotic drugs used in the susceptibility testing. In the labeling of every antibiotic class, a statement is or will be included that the susceptibility of the drug is determined by the class.

Physicians with knowledge that a particular class of antibiotics is effective against a pathogen can select the antibiotic from that class that best suits the individual needs of the patient. The advantages of using representative antibiotic discs include: (1) Saving of valuable laboratory time and expediting of results to physicians, because fewer individual discs are used in testing; (2) promotion of testing of a wider number of antibiotic drug classes; and (3) reduction of the possibility of an antibiotic in one class being used in treatment to the exclusion of another antibiotic or an antibiotic in another class which may be more appropriate. Based on consideration of these and other factors, and the overriding need for adequate and orderly diagnostic procedures for the use of antibiotic discs in testing the susceptibility of microorganisms to antibiotics, the Commissioner concludes that it is essential to the public interest that only those antibiotic discs representative of a class of antibiotics be certified. He further concludes that there is no provision in the law that makes it mandatory that discs be certified for the individual antibiotics in a class.

6. Comments were received that provisions should be made for antibiotic discs for use in differential isolation and identification of microorganisms. Two such discs are currently in use: (1) A disc containing 0.04 unit of bacitracin for presumptive identification of Group A streptococci, and (2) a disc containing 100 units of nystatin for suppression of overgrowth of molds and yeasts in the primary isolation of mixed bacterial cultures. The Commissioner considers this comment to be appropriate, and a new section has been added listing those antibiotic discs used in isolation of microorganisms or in determining their taxonomy and for exempting them from batch certification.

7. A comment was filed with data demonstrating a need for separate interpretations when using a clindamycin disc to simultaneously predict susceptibility to both clindamycin and lincomycin. The Commissioner concludes that separate interpretations are appropriate and such a statement has been added in § 147.2 (c) (2) in the Standardized Antibiotic Disc Susceptibility Test under *E. Interpretation of Zone Sizes*.

8. Responses were receiving questioning the need for both polymyxin B and colistin discs as representative of the same polymyxin class of antibiotics. The Commissioner finds that further studies are needed to develop a common susceptibility disc for this class, and notes that some such studies are in progress. The Commissioner, therefore, concludes that until a suitable single disc is developed, the listing of both polymyxin B and colistin is necessary.

RULES AND REGULATIONS

9. Requests were received to shorten the required vial label statement for antibiotic susceptibility discs to read "For laboratory use only". The Commissioner considers the statement "For laboratory use only" as adequate to reflect the intended use of these discs and § 147.2(c) (1) (iv) has been revised to provide for the shorter statement.

10. Data were presented to show that the 30 microgram cephalothin disc cannot be relied upon to detect resistance of methicillin-resistant staphylococci to cephalosporin class antibiotics. The Commissioner finds that a statement in the labeling is necessary to make known the limitations of the cephalothin disc. Therefore, such information is included in footnote (2) following the table in the section entitled: *E. Interpretation of Zone Sizes of § 147.2(c) (2)*.

11. Comment has been received that the proposed regulation appears to preclude the use of other procedures to determine antibiotic susceptibility. As outlined in the proposal, promulgation of this regulation is intended to help assure proper use of certified antibiotic discs. It is not intended to restrict the selection of methods or equipment used to test antibiotic susceptibility. Other methods (e.g., dilution techniques utilizing antibiotic powders) are recognized by the scientific community and the Food and Drug Administration. However, if modification of the disc method or if other methods are used, the results should be comparable to those obtained by the standardized method described in these regulations.

12. A number of comments concerned recommendations for a variety of technical changes in the Standardized Antibiotic Disc Susceptibility Test. All such recommendations were carefully considered and many have been incorporated in the order.

The Commissioner of Food and Drugs, having considered the comments received and other relevant material including recommendations from the Advisory Committee on Anti-infective Drugs, concludes the following:

1. The following named antibiotic and antibiotic-class discs are effective for use in susceptibility testing:

Name of Disc	Content of antibiotic in micrograms (mcg.) or units per disc
Ampicillin-class disc. ¹	10 mcg. ampicillin.
Bacitracin disc--	10 units bacitracin.
Carbenicillin disc.	50 mcg. carbenicillin.
Cephalosporin-class disc. ²	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. ³	2 mcg. clindamycin.
Neomycin disc--	30 mcg. neomycin.
Novobiocin disc--	30 mcg. novobiocin.
Oleandomycin disc. ⁴	15 mcg. oleandomycin.

Name of Disc	Content of antibiotic in micrograms (mcg.) or units per disc
Penicillin-class disc. ⁵	10 units penicillin G.
Penicillinase-resistant penicillin class disc. ⁶	5 mcg. methicillin.
Polymyxin B disc.	300 units polymyxin B.
Rifampin disc----	5 mcg. rifampin.
Streptomycin-class disc.	10 mcg. streptomycin.
Tetracycline-class disc. ⁷	30 mcg. tetracycline.
Vancomycin disc.	30 mcg. vancomycin.

¹ For determining susceptibility to ampicillin and hetacillin.
² For determining susceptibility to cephalothin, cephaloridine, cephaloglycin, and cephalixin.
³ For determining susceptibility to lincomycin and clindamycin.
⁴ For determining susceptibility to oleandomycin and troleandomycin.
⁵ For determining susceptibility to penicillin G, phenoxymethyl penicillin, and phenethicillin.
⁶ For determining susceptibility to methicillin, oxacillin, nafcillin, cloxacillin, and dicloxacillin.
⁷ For determining susceptibility to tetracycline, oxytetracycline, methacycline, doxycycline, demeclocycline, minocycline, rolitetracycline, and chlortetracycline.

2. The rifampin disc, having been found effective since the proposal was published and now being certified, is included in this order.

3. Data have been received regarding the expected zone sizes produced by the clindamycin disc for the two control cultures. Accordingly, the table under *F. Reference Organisms of the Standardized Disc Susceptibility Test* (§ 147.2(c) (2)) is amended to include clindamycin.

4. There is a need for a standardized antibiotic disc susceptibility test. A description of such method is required as part of the labeling of all antibiotic susceptibility discs subject to certification.

Therefore, pursuant to provisions of the Federal Food, Drug, and Cosmetic Act (secs. 502, 507, 52 Stat. 1050-51, as amended, 59 Stat. 463, as amended; 21 U.S.C. 352, 357) and under authority delegated to the Commissioner (21 CFR 2.120), Parts 144 and 147 are amended as follows:

1. Part 144 is amended by adding the following new section:

Antibiotic	Volume of suspension added to each 100 ml. of seed agar used for test	Suspension number	Medium	
			Base layer	Seed layer
Cephalothin	1.0	10	E	A

v. By alphabetically inserting a new item in the table in paragraph (d), as follows:

Antibiotic	Solvent	Standard curve (antibiotic concentration per disc)
Cephalothin	50 percent methyl alcohol	15.0, 21.2, 30.0, 42.4, 60.0

§ 144.17 Antibiotic drugs for isolation and differentiation of microorganisms in clinical use.

Antibiotic drugs subject to section 502(1) if such drugs are:

(a) Paper discs impregnated with antibiotics in the amounts listed in the following table:

Antibiotic	Content per Disc
Bacitracin	0.04 unit.
Nystatin	100 units.

(b) Packaged in a container bearing on its label or labeling the following:

- (1) On the outside wrapper or container and the immediate container:
 - (i) The batch mark.
 - (ii) The potency of each disc in the batch.
 - (iii) The expiration date as prescribed under § 148.3(a) (3) of this chapter.
- (iv) The statement: Not for Susceptibility Testing.
- (2) On the labeling within or attached to the package: Adequate directions for use.

2. Part 147 is amended in § 147.1 as follows:

§ 147.1 [Amended]

a. In § 147.1:
 i. By revising the section heading to read as follows: *§ 147.1 Antibiotic susceptibility tests and methods of assay; potency.*

ii. By deleting the following items from the table in paragraph (c) (3): Carbomycin (hydrochloride), chlortetracycline (hydrochloride), cloxacillin (hydrochloride), demeclocycline (hydrochloride), dihydrostreptomycin (sulfate), doxycycline, lincomycin (hydrochloride), methacycline (hydrochloride), nystatin, oxytetracycline (hydrochloride), phenethicillin potassium, ristocetin, sodium cephalothin, sodium nafcillin, sodium oxacillin, and viomycin (sulfate).

iii. By deleting the following items from the table in paragraph (d): Carbomycin (hydrochloride), chlortetracycline (hydrochloride), cloxacillin, demeclocycline (hydrochloride), dicloxacillin, dihydrostreptomycin, doxycycline, lincomycin (hydrochloride), methacycline (hydrochloride), nystatin, oxytetracycline (hydrochloride), phenethicillin potassium, ristocetin, sodium cephalothin, sodium nafcillin, sodium oxacillin, and viomycin (sulfate).

iv. By alphabetically inserting a new item in the table in paragraph (c) (3), as follows:

vi. By changing the fourth sentence of paragraph (e) (1) to read "Incubate the plates overnight at 32°-35° C., except if cephalothin, colistin, novobiocin, or myxlin, the incubation temperature is 37° C."

vii. By changing the heading of subparagraph (3) in paragraph (e) to read "Individual discs with diameters larger than one-fourth inch but no larger than three-eighths inch for use in impregnating culture media".

viii. By deleting paragraph (e) (4).

b. By revising the section heading and text of § 147.2 to read as follows:

§ 147.2 Antibiotic susceptibility discs; certification procedure.

(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.
- Amphotericin: 10 units.
- Chloramphenicol: 50 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.
- Vancomycin: 30 mcg.

The standard discs used to determine the potency shall be made of paper as described in § 147.1(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 § 146.10 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.
Bactracin disc.	10 units bacitracin.
Carbenicillin disc.	50 mcg. carbenicillin.
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.
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:

quate 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 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.

RULES AND REGULATIONS

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 evenly 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 micro-organisms 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.....	21-23	24 or more.
Ampicillin ¹ when testing <i>Haemophilus</i> species.....	10 mcg.....	19 or less.....		20 or more.
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.
Cephalothin when reporting susceptibility to cephalothin, cephaloridine, and cephalixin.	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. 21
Colistin.....	10 mcg.....	8 or less.....	9-10	11 or more. ⁴
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.....	13-18	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.....	21-22	23 or more. 29
Penicillin G when testing other micro-organisms ⁸	10 units.....	11 or less.....	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.
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.

⁷ Not applicable to medium that contains blood.

⁸ The oleandomycin disc is used for testing susceptibility to oleandomycin and troleandomycin.

⁹ 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.

¹⁰ This category includes some organisms such as enterococci and gram-negative bacilli that may cause 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.

¹¹ The tetracycline disc is used for testing susceptibility to all tetracyclines; that is, chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, rolitetracycline, minocycline, and tetracycline.

F. REFERENCE ORGANISMS

1. Maintain stock cultures of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).

2. Test these reference organisms daily by the above procedure using antibiotic discs

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

3. 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 25922- ATCC 25922
		With <i>S. aureus</i> ATCC 25922	With <i>E. coli</i> ATCC 25922	
Ampicillin.....	10 mcg.....	24-35	15-20	7-17
Bacitracin.....	10 units.....	17-22		
Cephalothin.....	30 mcg.....	25-37	18-23	5-16
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.....	19-23		
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
Tetracycline.....	30 mcg.....	19-28	18-25	0-6
Vancomycin.....	30 mcg.....	15-19		

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.

§§ 147.3, 147.4 [Revoked]

C. By revoking § 147.3 *Antibiotic-dehydrated media-triphenyltetrazolium chloride sensitivity discs; tests and methods of assay* and § 147.4 *Antibiotic-dehydrated media-triphenyltetrazolium chloride sensitivity discs; certification pro-*

Any person who will be adversely affected by the removal of any such drugs from the market may file objections to this order and request a hearing, showing reasonable grounds therefor. The statement of reasonable grounds and request for a hearing shall be submitted in writing within 30 days after publication hereof in the FEDERAL REGISTER, shall state the reasons why the antibiotic drug regulations should not be so amended, and shall include a well organized and full factual analysis of the clinical and other investigational data the objector is prepared to present in support of his objections.

A request for a hearing may not rest upon mere allegations or denials, but must set forth specific facts showing that a genuine and substantial issue of fact requires a hearing. When it clearly appears from the data incorporated into or referred to by the objections and from the factual analysis in the request for a hearing that no genuine issue of fact precludes the action taken by this order, the Commissioner will enter an order stating his findings and conclusions on such data.

If a hearing is requested and justified by the objections, the issues will be defined and a hearing examiner named to conduct the hearing. The provisions of Subpart F of 21 CFR Part 2 shall apply to such hearing, except as modified by 21 CFR 146.1(f), and to judicial review

in accordance with section 701 (f) and (g) of the Federal Food, Drug, and Cosmetic Act (35 F.R. 7250, May 8, 1970).

Objections and requests for a hearing should be filed (preferably in quintuplicate) with the Hearing Clerk, Department of Health, Education, and Welfare, Room 6-88, 5600 Fishers Lane, Rockville, Md. 20852. Received objections and requests for a hearing may be seen in the above office during regular business hours, Monday through Friday.

Effective date. This order shall become effective 40 days after its date of publication in the FEDERAL REGISTER. If objections are filed, the effective date will be extended for ruling thereon. In so ruling, the Commissioner will specify another effective date.

(Secs. 502, 507, 52 Stat. 1050-51, as amended, 59 Stat. 463, as amended; 21 U.S.C. 352, 357)

Dated: September 22, 1972.

SAM D. FINE,
Associate Commissioner
for Compliance.

[FR Doc.72-16571 Filed 9-29-72; 8:45 am]

SUBCHAPTER D—HAZARDOUS SUBSTANCES
PART 191—HAZARDOUS SUBSTANCES: DEFINITIONS AND PROCEDURAL AND INTERPRETATIVE REGULATIONS

Confirmation of Effective Date of Order Classifying Asbestos-Containing Garments for General Use as Banned Hazardous Substances

In the matter of classifying general-use garments containing asbestos as banned hazardous substances because inhalation of asbestos fibers can cause lung cancer, mesothelioma, and lung fibrosis:

Pursuant to provisions of the Federal Hazardous Substances Act (sec. 2(q)(1)(B), (2), 74 Stat. 374, as amended, 80 Stat. 1304-05; 15 U.S.C. 1261) and of the Federal Food, Drug, and Cosmetic Act (sec. 701(e), 52 Stat. 1055, as amended; 21 U.S.C. 371(e)), and under authority delegated to the Commissioner of Food and Drugs (21 CFR 2.120), notice is given that no objections were filed

to the order in the above-identified matter published in the FEDERAL REGISTER of July 26, 1972 (37 F.R. 14872). Accordingly, the regulation promulgated thereby (21 CFR 191.9(a)(7)) shall become effective September 24, 1972.

Dated: September 25, 1972.

SAM D. FINE,
Associate Commissioner
for Compliance.

[FR Doc.72-16653 Filed 9-29-72; 8:46 am]

Title 5—ADMINISTRATIVE PERSONNEL

Chapter I—Civil Service Commission
PART 213—EXCEPTED SERVICE

U.S. Government Printing Office

Section 213.3152 is amended to show that positions in the printing trades, when filled by students majoring in printing technology employed under a cooperative education agreement with the Washington Technical Institute, are excepted under Schedule A.

Effective on publication in the FEDERAL REGISTER (9-30-72), § 213.3152(b) is added as set out below.

§ 213.3152 U.S. Government Printing Office.

(b) Positions in the printing trades when filled by students majoring in printing technology employed under a cooperative education agreement with the Washington Technical Institute.

(5 U.S.C. secs. 3301, 3302, E.O. 10577; 3 CFR 1954-58 Comp., p. 218)

UNITED STATES CIVIL SERVICE COMMISSION,

[SEAL] JAMES C. SPRY,
Executive Assistant
to the Commissioners.

[FR Doc.72-16696 Filed 9-29-72; 8:49 am]

PART 213—EXCEPTED SERVICE

Department of Defense

Section 213.3306 is amended to reflect the following title change: From Private Secretary to the Deputy Director, Defense Research and Engineering (Strategic and Space Systems), to Private Secretary to the Deputy Director, Defense Research and Engineering (Strategic Systems).

Effective on publication in the FEDERAL REGISTER (9-30-72), § 213.3306(a)(2) is amended as set out below.

§ 213.3306 Department of Defense.

- (a) *Office of the Secretary.* * * *
- (2) One Private Secretary to the Deputy Secretary of Defense and one Private Secretary to each of the following: the Director of Defense Research and Engineering; the Principal Deputy Director of Defense Research and Engineering;