

at daily and weekly interveral using freshly prepared standard disc for comparison.

(5) *Assay*—(i) *Individual discs*. On each of three plates prepared as directed in paragraph (b)(3) of this section, place standard disc and two or more discs from each batch to be tested. The standard disc and the sample discs are placed on the plates in a circular pattern with random arrangement, with no disc being closer than 24 millimeters (on centers) to any other disc. Discs are placed on the plates within as short a period of time as possible (not to exceed 3 minutes per plate) and tapped gently to ensure an even seal. After incubation as directed in paragraph (b)(3) of this section, measure the diameter of each circle of inhibition as accurately as possible. (In most cases, it is possible to estimate diameters to the nearest 0.1 millimeter).

(ii) *Estimation of potency*. Determine the logarithm of each dose of standard (x values) and the mean zone diameter for each dose of standard (y values). Using the three values of x and the three corresponding values of y, calculate Σx , Σx^2 , $(\Sigma x)^2$, Σy , and Σxy . Calculate the regression coefficient (slope, b) and the Y-intercept (a) of the standard response line by using the following equations:

$$b = \frac{n\Sigma xy - (\Sigma x)(\Sigma y)}{n\Sigma x^2 - (\Sigma x)^2}$$

$$a = \frac{\Sigma y - b\Sigma x}{n}$$

where n = the number of standard doses.

Determine the zone diameter (Y) for each sample disc being tested. Using the regression equation

$$X = \text{antilog} \frac{Y - a}{b}$$

calculate the concentration (X) for the mean response (Y) of the sample discs.

(6) *Potency*. The potency of the batch is satisfactory if the mean result obtained for the batch is not less than 85 percent and not more than 150 percent of that represented.

[45 FR 20668, Apr. 6, 1979]

§ 460.15 Streptomycin sulfate discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Streptomycin sulfate discs for use in culture media are paper discs intended for impregnation of culture media in the sensitivity testing of mycobacteria. They conform to all requirements and to all procedures prescribed by § 460.1(a) for antibiotic sensitivity discs, except that each disc shall contain streptomycin sulfate equivalent to 10, 25, 50, or 500 micrograms of streptomycin.

(2) *Packaging*. It shall be packaged in accordance with the requirements of § 460.1(b).

(3) *Labeling*. In addition to complying with the requirements of § 460.1(c) of this chapter, the labeling shall also bear information indicating that the discs are for use in culture media for the sensitivity testing of mycobacteria and not for use in ordinary sensitivity disc plate tests.

(4) *Requests for certification; samples*. Requests for certification shall comply with § 460.1(d).

(b) *Tests and methods of assay; potency*. Proceed as directed in § 460.6 for the assay of streptomycin sulfate discs, except that:

(1) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 10, 25, or 50 micrograms of streptomycin, the control discs shall be made to contain the equivalent of 6.25, 12.5, 25, 50, and 100 micrograms of streptomycin per disc.

(2) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 500 micrograms of streptomycin:

(i) To each 100 milliliters of seed agar used for the test add 2.0 milliliters of suspension number 11.

(ii) The control discs shall be made to contain the equivalent of 50, 100, 200, 400, and 800 micrograms of streptomycin per disc.

§ 460.16 Rifampin discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rifampin discs for use in culture media are paper discs intended for impregnation of culture media in

the susceptibility testing of mycobacteria. They conform to all requirements and to all procedures prescribed by § 460.1(a) for antibiotic susceptibility discs, except that each disc shall contain 25 micrograms of rifampin activity.

(2) *Packaging.* It shall be packaged in accordance with the requirements of § 460.1(b).

(3) *Labeling.* In addition to complying with the requirements of § 460.1(c), the labeling shall also bear information indicating that the discs are for use in culture media for the susceptibility testing of mycobacteria and not for use in susceptibility tests of other microorganisms as described in § 460.1(c)(2).

(4) *Requests for certification; samples.* Requests for certification shall comply with § 460.1(d), except an accurately representative sample of the batch shall consist of one disc for each 5,000 in the batch, but in no case less than 100 discs collected by taking single discs at such intervals throughout the entire time of manufacturing the batch that the quantities manufactured during the intervals are approximately equal.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 460.6.

[41 FR 53476, Dec. 7, 1976]

Subpart B—Susceptibility Powders

§ 460.25 Bacitracin diagnostic sensitivity powder.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Bacitracin diagnostic sensitivity powder is bacitracin, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to bacitracin. Each vial contains 2,000 units of bacitracin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The bacitracin used conforms to the standards prescribed by § 448.10a(a)(1) (i), (v), and (vi) of this chapter. Each other

substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(a) The statement “For laboratory diagnostic use only.”

(b) The statement “Sterile.”

(c) The batch mark.

(d) The number of units of bacitracin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The bacitracin used in making the batch for potency, moisture, and pH.

(b) The batch for potency sterility, loss on drying, and pH.

(ii) Samples required:

(a) The bacitracin used in making the batch: 6 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 20 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed reference concentration.