

§ 436.106 Microbiological turbidimetric assay.

Using the sample solution prepared as described in the section for the particular antibiotic to be tested, proceed as described in paragraphs (a), (b), and (c) of this section.

(a) *Preparation of working standard stock solutions and standard response line solutions.* For each antibiotic listed in the table in this paragraph, select the working standard, drying conditions, solvent(s), concentrations, and storage time for the standard solutions and proceed as follows: If necessary, dry

the working standard as described in § 436.200; dissolve and dilute an accurately weighed portion to the proper concentration for the working standard stock solution. Store the working standard stock solution under refrigeration and do not use longer than the recommended storage time. Prepare the proper concentrations for the standard response line solutions by further diluting an aliquot of the working standard stock solution. The reference concentration of the assay is the mid concentration of the standard response line.

| Antibiotic | Working standard stock solutions | | | | Standard response line concentrations | | |
|-----------------------|---|------------------------------------|---|--|---------------------------------------|---|---|
| | Drying conditions (method number assigned in § 436.200) | Initial solvent | Diluent (solution number as listed in § 436.101(a)) | Final concentration units or milligrams per milliliter | Storage time under refrigeration | Diluent (solution number as listed in § 436.101(a)) | Final concentrations—units or micrograms of antibiotic activity per milliliter |
| Amikacin | Not dried | | Distilled water | 1 mg | 2 weeks | Distilled water | 8.0, 8.9, 10.0, 11.2, 12.5 µg. |
| Candidin ¹ | 6 | | Dimethyl sulfoxide | 1 mg | Use same day | Distilled water | 0.030, 0.043, 0.060, 0.085, 0.120 µg. (Prepare standard response line simultaneously with the sample solution.) |
| Capreomycin | 5 | Distilled water | | 1 mg | 7 days | Distilled water | 80.0, 89.0, 100.0, 112.0, 125.0 µg. |
| Chloramphenicol | Not dried | Ethyl alcohol (10,000 µg. per ml.) | Distilled water | 1 mg | 1 month | Distilled water | 2.00, 2.24, 2.50, 2.80, 3.12 µg. |
| Chlortetracycline | Not dried | | 0.01N HCl | 1 mg | 4 days | Distilled water | 0.048, 0.054, 0.060, 0.067, 0.075 µg. |
| Cycloserine | 1 | | Distilled water | 1 mg | 1 month | Distilled water | 40.0, 44.5, 50.0, 56.0, 62.5 µg. |
| Demeclocycline | 1 | | 0.1N HCl | 1 mg | 4 days | Distilled water | 0.080, 0.089, 0.100, 0.112, 0.125 µg. |
| Dihydrostreptomycin | 5 | | Distilled water | 1 mg | 30 days | Distilled water | 24.0, 26.8, 30.0, 33.5, 37.5 µg. |
| Doxycycline | Not dried | | 0.1N HCl | 1 mg | 5 days | Distilled water | 0.080, 0.089, 0.100, 0.112, 0.125 µg. |
| Gramicidin | 1 | | alcohol U.S.P. XX | 1 mg | 30 days | alcohol U.S.P. XX | 0.032, 0.0356, 0.040, 0.0448, 0.050 µg. |
| Kanamycin | Not dried | | Distilled water | 1 mg | 1 month | Distilled water | 8.0, 8.9, 10.0, 11.2, 12.5 µg. |
| Lincosmycin | Not dried | | Distilled water | 1 mg | 1 month | Distilled water | 0.400, 0.447, 0.500, 0.559, 0.625 µg. |
| Meclocycline | Not dried | | 13 | 1 mg | Use same day | Distilled water | 0.048, 0.054, 0.06, 0.067, 0.075 µg. |
| Methacycline | 1 | | Distilled water | 1 mg | 7 days | Distilled water | 0.048, 0.054, 0.060, 0.067, 0.075 µg. |
| Oxytetracycline | Not dried | | 0.1N HCl | 1 mg | 4 days | Distilled water | 0.192, 0.215, 0.240, 0.268, 0.300 µg. |
| Rolifetracycline | 1 | | Distilled water | 1 mg | 1 day | Distilled water | 0.192, 0.215, 0.240, 0.268, 0.300 µg. |
| Spectinomycin | Not dried | | Distilled water | 1 mg | 1 month | Distilled water | 24.0, 26.8, 30.0, 33.5, 37.5 µg. |
| Streptomycin | 1 | | Distilled water | 1 mg | 30 days | Distilled water | 24.0, 26.8, 30.0, 33.5, 37.5 µg. |
| Tetracycline | Not dried | | 0.1N HCl | 1 mg | 1 day | Distilled water | 0.192, 0.215, 0.240, 0.268, 0.300 µg. |
| Tobramycin | Not dried | | Distilled water | 1 mg | 2 weeks | Distilled water | 2.00, 2.236, 2.5, 2.795, 3.125 µg. |
| Troleandomycin | 1 | | 15 | 1 mg | Use same day | Distilled water | 20.0, 22.25, 25.0, 28.0, 31.25 µg. |
| Tyrosin ² | | | | | | | |

¹ Use sterile equipment for all stages of this assay.

² The gramicidin working standard and the gramicidin standard response line concentrations are used for the assay of tyrothricin.

(b) *Procedure for assay.* For each antibiotic listed in the table in this paragraph, select the test organism (as listed in §436.103(a)), nutrient broth (as listed by medium number in §436.102(b)), and suggested inoculum and proceed as follows: Place 1.0 milliliter (or 0.1 milliliter in the case of gramicidin and tyrothricin) of each concentration of the standard response line (prepare as described in paragraph (a) of this section) and of the sample solution in each set of three replicate tubes (as described in §436.100(b)(1)). Fifteen tubes are used for the five-point standard response line and three for each sample. To each tube add 9 milliliters of the inoculated broth and place immediately in a water bath at the appropriate temperature for 2 to 4

hours. The exact length of the incubation period should be determined by observation of growth in the reference concentration tube of the standard. Remove the tubes from the water bath and add 0.5 milliliter of a 12-percent formaldehyde solution to each tube. Determine the absorbance value of each tube in a suitable photoelectric colorimeter, at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with an uninoculated blank composed of the same amounts of nutrient broth and formaldehyde used in the assay.

NOTE: The amount of working standard and sample solutions may be reduced as long as all other solutions used are reduced proportionately.

| Antibiotic | Test organism | Medium (nutrient broth) | Suggested volume of standardized inoculum to be added to each 100 milliliters of medium (nutrient broth) | Incubation temperature |
|-------------------------------|---------------|-------------------------|--|------------------------|
| Amikacin | A | 3 | 0.1 | 36–37.5 |
| Candicidin ¹ | E | 13 | 0.2 | 27–29 |
| Capreomycin | I | 3 | 0.05 | 36–37.5 |
| Chloramphenicol | J | 3 | 0.7 | 36–37.5 |
| Chlortetracycline | A | 3 | 0.1 | 36–37.5 |
| Cycloserine | A | 3 | 0.4 | 36–37.5 |
| Demeclocycline | A | 3 | 0.1 | 36–37.5 |
| Dihydrostreptomycin | I | 3 | 0.1 | 36–37.5 |
| Doxycycline | A | 3 | 0.1 | 36–37.5 |
| Gramicidin | K | 3 | 1.0 | 36–37.5 |
| Kanamycin | A | 3 | 0.2 | 36–37.5 |
| Lincomycin | A | 3 | 0.1 | 36–37.5 |
| Meclocycline | A | 3 | 0.2 | 36–37.5 |
| Methacycline | A | 3 | 0.1 | 36–37.5 |
| Oxytetracycline | A | 3 | 0.1 | 36–37.5 |
| Rolitetra-cycline | A | 3 | 0.1 | 36–37.5 |
| Spectinomycin | J | 3 | 0.1 | 36–37.5 |
| Streptomycin | I | 3 | 0.1 | 36–37.5 |
| Tetracycline | A | 3 | 0.1 | 36–37.5 |
| Tobramycin | A | 3 | 0.15 | 36–37.5 |
| Troleandomycin | I | 3 | 0.1 | 36–37.5 |
| Tyrothricin | K | 3 | 1.0 | 36–37.5 |

¹ Use sterile equipment for all stages of this assay.

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

where:

L=Calculated absorbance value for the lowest concentration of the standard response line.

H=Calculated absorbance value for the highest concentration of the standard response line.

a, b, c, d, e—Average absorbance values for each concentration of the standard response line, lowest to the highest, respectively.

(c) *Estimation of potency.* To prepare the standard response line, plot the average absorbance values for each concentration of the standard response line on one-cycle semilogarithmic graph paper with the absorbance values on the arithmetic scale and concentrations on the logarithmic scale. The response line is drawn either through these points by inspection or through points plotted for highest and lowest absorbance values obtained by means of the following equations.

To estimate the potency of the sample, average the absorbance values for the sample and determine the antibiotic concentration from the standard response line. Multiply the concentration by the appropriate dilution factor to obtain the antibiotic content of the sample.

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Subpart E—General Chemical Tests for Antibiotics

§ 436.200 Loss on drying.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* In an atmosphere of about 10 percent relative humidity, grind the sample, if necessary, to obtain a fine powder. When tablets, troches, or capsules are to be tested, use four tablets, troches, or capsules in preparing the sample. Transfer about 100 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel.

Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent, such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent of loss.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use a tared weighing bottle or weighing tube equipped with a capillary-tube stopper, the capillary having an inside diameter of 0.20–0.25 millimeter, and place it in a vacuum oven without removing the stopper.

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 110° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 2 hours.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 100° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 25° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(h) *Method 8.* Proceed as directed in paragraph (a) of this section, except transfer approximately 300 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper; dry the sample at a temperature of 25 ° C and a pressure of 5 millimeters of mercury or less for 4 hours, and then dry the sample at a temperature of 100 °C and a pressure of 5 millimeters of mercury or less for 3 additional hours.

(i) *Method 9.* Use a suitable thermogravimetric apparatus prepared for vacuum operation. Rapidly and thoroughly grind a portion of the sample and promptly transfer 5 to 10 milligrams to the sample pan. Place the system under vacuum and allow it to come to equilibrium before heating. Obtain an accurate sample weight and