

(3) *Bacterial membrane filter method for ophthalmic ointments*—(i) *Ointments that do not contain penicillin.* From each of 10 immediate containers aseptically transfer 0.1 gram of the product into a sterile 250-milliliter flask containing 100 milliliters of diluting fluid E which has previously been heated to a temperature of 47° C. Repeat the process, using 10 additional containers. Swirl both of the flasks to dissolve the ointment. Immediately aseptically filter each solution through a separate bacteriological membrane filter previously moistened with approximately 0.2 milliliter of medium K. Filter all air entering the system through air filters capable of removing microorganisms. Remove any residual antibiotic from the membranes by rinsing each filter five times with 100 milliliters of medium K. The membranes should be covered with fluid throughout each step of the filtration procedure until the end of the last filtering step. By means of a sterile circular blade, paper punch, or other suitable sterile device, cut a circular portion (approximately 17.5 millimeters in diameter) from the center of the filtering area of each membrane. Transfer the center portion of the filtering area of each filter to a sterile test tube 38 millimeters × 200 millimeters (outside dimensions) containing 90 milliliters ± 10 milliliters of sterile medium I. Incubate the tube for 7 days at 30° C. to 32° C. Using sterile forceps transfer the outer portion of each filter to a similar test tube containing 90 milliliters ± 10 milliliters of sterile medium J. Incubate this tube for 7 days at 22° C. to 25° C.

(ii) *Ointments containing penicillin.* Proceed as directed in paragraph (e)(3)(i) of this section, except in lieu of sterile medium I use sterile medium L for the center portion of the filtering area of each filter and in lieu of sterile medium J use sterile medium M for the remaining outer portion of each filter.

(f) *Evaluation of results*—(1) *Bacterial membrane-filter method.* The batch, or the part of the batch represented by a particular filling operation meets the requirements of the test if no sample tube shows growth. If growth is observed in any sample tube, run a second test in the appropriate medium, except perform it in duplicate, using 40

immediate containers. If in the original test, growth is observed in only one of the two media, test both portions of the cut filter membrane by placing each into a separate tube of the same medium. The batch meets the requirements if no tube on the second test shows growth. If growth is observed in any of the control tubes as well as in the sample tubes in either the original or the second test such test is invalid and must be performed again. In any event, further tests may be justified if there is sufficient reason to believe that the results obtained in the first and second tests may not be valid. In such instances, the batch is satisfactory if on the final test no tube shows growth.

(2) *Direct method.* The batch, or the part of the batch represented by a particular filling operation, meets the requirements of the test if no tube shows growth after incubation. If growth is observed in any sample tube, run a second test in the appropriate medium using 40 immediate containers. The batch is satisfactory if, on the second test, no tube shows growth. If growth is observed in any of the control tubes (except inoculated tubes, if the sample is penicillin) as well as in the sample tubes in either the original or the second test, such test is invalid and must be performed again. In any event, further tests may be justified if there is sufficient reason to believe that the results obtained on the first and second tests may not be valid. In such instances the batch is satisfactory if in the final test no tube shows growth.

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Subpart C—Biological Test Methods

§ 436.31 Equipment and diluents for use in biological testing.

(a) *Equipment*—(1) *Temperature-measuring devices.* Use an accurate clinical

thermometer or any other temperature-measuring device of equal sensitivity that has been tested to determine the time necessary to reach the maximum reading.

(2) *Pyrogen-free glassware.* Render all glassware free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method.

(3) *Pyrogen-free syringes and needles.* Render all syringes and needles free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method.

(4) *Pyrogen-free sodium chloride.* Heat sodium chloride for not less than 2 hours at 200° C.

(5) *Pyrogen-free sodium carbonate.* Heat anhydrous sodium carbonate for not less than 4 hours at 170° C.

(b) *Diluents.* (1) Diluent 1 (pyrogen-free water): Prepare pyrogen-free water by collecting freshly distilled water and sterilizing it in an autoclave at 121° C. for not less than 20 minutes. Pyrogen-free water meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 10 milliliters per kilogram are administered as described in § 436.32(a)(2). In testing water for the absence of pyrogens, the aliquot to be tested is made isotonic by the addition of pyrogen-free sodium chloride.

(2) Diluent 2 (pyrogen-free saline solution): Prepare an isotonic solution of sodium chloride by dissolving 9.0 grams of pyrogen-free sodium chloride (prepared as described in § 436.31(a)(4)) in pyrogen-free, distilled water (diluent 1) to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for not less than 20 minutes. Pyrogen-free saline solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 10 milliliters per kilogram are administered as described in § 436.32(a)(2).

(3) Diluent 3 (sterile distilled water): Prepare freshly distilled water. Sterilize in an autoclave at 121° C. for 20 minutes.

(4) Diluent 4 (sterile saline solution): Dissolve 9.0 grams of sodium chloride in distilled water to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for 20 minutes.

(5) Diluent 5 (10 percent gum acacia): Dissolve 10 grams of gum acacia in ap-

proximately 50 milliliters of distilled water. Allow to stand overnight at room temperature and dilute to 100 milliliters with distilled water. Filter through cotton. Store under refrigeration.

(6) Diluent 6 (0.5 percent gum acacia in distilled water). 11132

(7) Diluent 7 (1.0N hydrochloric acid).

(8) Diluent 8 (0.1N hydrochloric acid).

(9) Diluent 9 (0.05N sodium hydroxide).

(10) Diluent 10 (1 percent U.S.P. methylcellulose (4,000 centipoises) solution): Dissolve 1 gram of U.S.P. methylcellulose (4,000 centipoises) in 100 milliliters of distilled water. Allow to stand overnight at room temperature or until solution is complete. Store under refrigeration.

(11) Diluent 11 (0.12N sodium hydroxide).

(12) Diluent 12 (0.5 percent methylcellulose (4,000 centipoises) in distilled water). Proceed as directed in paragraph (b)(10) of this section, except use 0.5 gram of methylcellulose (4,000 centipoises).

(13) Diluent 13 (pyrogen-free sodium carbonate solution). Dissolve 25.6 grams of anhydrous pyrogen-free sodium carbonate (prepared as described in paragraph (a)(5) of this section) in 1,000 milliliters pyrogen-free, distilled water (diluent 1). Pyrogen-free, sodium carbonate solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 1.0 milliliter per kilogram is administered as described in § 436.32(a)(2).

(14) Diluent 14 (0.07M sterile sodium carbonate solution). Dissolve 7.3 grams of sodium carbonate in distilled water to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for 20 minutes.

(15) Diluent 15 (pyrogen-free sodium carbonate solution): Dissolve 9.9 grams of anhydrous pyrogen-free sodium carbonate (prepared as directed in paragraph (a)(5) of this section) in 1,000 milliliters of pyrogen-free, distilled water (diluent 1). Pyrogen-free sodium carbonate solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 1.0 milliliter per kilogram is administered as described in § 436.32(a)(2).

(16) Diluent 16 (0.13M sterile pyrogen-free sodium carbonate solution). Dissolve 14.0 grams of anhydrous pyrogen-free sodium carbonate (prepared as described in paragraph (a)(5) of this section) in 1,000 milliliters pyrogen-free, distilled water. Sterilize in an autoclave at 121 °C for 20 minutes.

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§ 436.32 Pyrogen test.

(a) *Method 1*—(1) *Test animal*. Use healthy, mature rabbits weighing not less than 1,800 grams each that have maintained their weight on an antibiotic-free diet for at least 1 week under the environmental conditions specified in this section. House the animals individually in an area of uniform temperature ($\pm 3^\circ$ C.) and free from disturbances likely to excite them. Do not use animals for pyrogen tests more frequently than once every 48 hours or prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic. Before using an animal that has not been used for a test during the previous 2 weeks, condition it 1 to 3 days prior to pyrogen testing by conducting a sham test as directed in paragraph (a)(2) of this section, omitting the injection.

(2) *Procedure*. Using equipment and diluents described in § 436.31, as necessary, perform the test in an area where the animals are housed or under similar environmental conditions. On the day of the test: Withhold all food from the animals being used until after completion of the test, except that access to water may be allowed; and determine the "control temperature" of each animal by inserting the temperature-measuring device into the rectum of the test animal to a depth of not less than 7.5 centimeters and allowing sufficient time to reach a maximum temperature, as previously determined, before taking the reading. In any one test use only those animals whose control temperatures do not deviate by more than 1° C. from each other and do not use any animal with a temperature exceeding 39.8° C. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the in-

jection of the material is calculated. If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling. Warm the product to be tested to approximately 37° C. Dilute the sample with sterile, pyrogen-free saline (prepared as described in § 436.31(b)(2)) to the appropriate concentration specified in the individual section for each antibiotic to be tested. Inject a test dose of 1 milliliter of the diluted sample per kilogram of rabbit weight into an ear vein of each of three rabbits within 30 minutes subsequent to the control temperature reading. Record the temperature at 1, 2, and 3 hours subsequent to the injection.

(3) *Evaluation*. If no rabbit shows an individual rise in temperature of 0.6° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.4° C., the sample meets the requirements for the absence of pyrogens. If one or two rabbits show a temperature rise of 0.6° C. or more, or if the sum of the temperature rises exceeds 1.4° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6° C. or more, and if the sum of the eight temperature rises does not exceed 3.7° C., the sample meets the requirements for the absence of pyrogens.

(b) *Method 2*. Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1).

(c) *Method 3*. Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(d) *Method 4*. Proceed as directed in paragraph (a) of this section, except inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(e) *Method 5*. Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.