

growth-promoting qualities. If any portion of a lot of commercial dehydrated medium is held for 90 days or longer after being so tested, it shall be retested before use. Two or more strains of micro-organisms that are exacting in their nutritive requirements shall be used. More than one dilution shall be used to demonstrate the adequacy of the medium to support the growth of a minimum number of micro-organisms.

(c) The sterility of the medium shall be confirmed by incubating an adequate number of test vessels and examining each for growth. Additional control may be used by incubation of representative uninoculated test vessels for the required incubation period during each test.

(d) A determination shall be made by the licensee for each biological product of the ratio of inoculum to medium which shall result in sufficient dilution of such product to prevent bacteriostatic and fungistatic activity. The determination may be made by tests on a representative biological product for each group of comparable products containing identical preservatives at equal or lower concentrations. Inhibitors or neutralizers of preservatives, approved by the Administrator, may be considered in determining the proper ratio.

[35 FR 16039, Oct. 13, 1970, as amended at 37 FR 2430, Feb. 1, 1972; 41 FR 27715, July 6, 1976; 56 FR 66784, Dec. 26, 1991]

**§ 113.26 Detection of viable bacteria and fungi except in live vaccine.**

Each serial and subserial of biological product except live vaccines shall be tested as prescribed in this section unless otherwise specified by the Administrator. When cell lines, primary cells, or ingredients of animal origin used in the preparation of a biological product are required to be free of viable bacteria and fungi, they shall also be tested as prescribed in this section.

(a) The media to be used shall be as follows:

(1) Fluid Thioglycollate Medium with 0.5 percent beef extract shall be used to test for bacteria in biological products containing clostridial toxoids, bacterins, and bacterin-toxoids.

(2) Fluid Thioglycollate Medium with or without 0.5 percent beef extract

shall be used to test for bacteria in biological products other than clostridial toxoids, bacterins, and bacterin-toxoids.

(3) Soybean-Casein Digest Medium shall be used to test biological products for fungi; provided, that Fluid Thioglycollate Medium without beef extract shall be substituted when testing biological products containing mercurial preservatives.

(b) Test procedure:

(1) Ten test vessels shall be used for each of two media selected in accordance with paragraph (a)(1), (a)(2), or (a)(3) of this section. Each test vessel shall contain sufficient medium to negate the bacteriostatic or fungistatic activity in the inoculum as determined in § 113.25(d).

(2) Inoculum:

(i) When completed product is tested, 10 final container samples from each serial and each subserial shall be tested. One ml from each sample shall be inoculated into a corresponding individual test vessel of culture medium: *Provided*, That, if each final container sample contains less than 2 ml, one-half of the contents shall be used as inoculum for each test vessel.

(ii) When cell lines, primary cells, or ingredients of animal origin are tested, at least a 20 ml test sample from each lot shall be tested. One ml shall be inoculated into each test vessel of medium.

(3) Incubation shall be for an observation period of 14 days at 30 °to 35 °C. to test for bacteria and 14 days at 20 °to 25 °C. to test for fungi.

(4) If the inoculum renders the medium turbid so that the absence of growth cannot be determined by visual examination, subcultures shall be made on the seventh to eleventh day from biological products prepared from clostridial toxoids, bacterins, and bacterin-toxoids and the third to seventh day for other biological products. Portions of the turbid medium in amounts of not less than 1.0 ml. shall be transferred to 20 to 25 ml. of fresh medium, and incubated the balance of the 14-day period.

(c) Examine the contents of all test vessels for macroscopic microbial growth during the incubation period.

When demonstrated by adequate controls to be invalid, the test may be repeated. For each set of test vessels representing a serial or subserial in a valid test, the following rules shall apply:

(1) If no growth is found in any test vessel, the serial or subserial meets the requirements of the test.

(2) If growth is found in any test vessel, one retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(3) If growth is found in any test vessel of the final test, the serial, subserial, or ingredients to be used in the preparation of a biological product, as the case may be, is unsatisfactory.

[35 FR 16039, Oct. 13, 1970, as amended at 37 FR 2430, Feb. 1, 1972; 39 FR 21042, June 18, 1974; 40 FR 758, Jan. 3, 1975; 40 FR 14084, Mar. 28, 1975; 56 FR 66784, Dec. 26, 1991]

**§ 113.27 Detection of extraneous viable bacteria and fungi in live vaccines.**

Unless otherwise specified by the Administrator or elsewhere exempted in this part, each serial and subserial of live vaccine and each lot of Master Seed Virus and Master Seed Bacteria shall be tested for extraneous viable bacteria and fungi as prescribed in this section. A Master Seed found unsatisfactory shall not be used in vaccine production and a serial found unsatisfactory shall not be released.

(a) *Live viral vaccines.* Each serial and subserial of live viral vaccine shall be tested for purity as prescribed in this paragraph. However, products of chicken embryo origin recommended for administration other than by parenteral injection may be tested as provided in paragraph (e) of this section.

(1) Soybean Casein Digest Medium shall be used.

(2) Ten final container samples from each serial and subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and desiccated vaccine shall be rehydrated as recommended on the label with accompanying diluent or with sterile purified water.

(4) To test for bacteria, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 120 ml of Soybean

Casein Digest Medium. Additional medium shall be used if the determination required in §113.25(d) indicates the need for a greater dilution of the product. Incubation shall be at 30 °to 35 °C for 14 days.

(5) To test for fungi, place 0.2 ml of vaccine from each final container sample into a corresponding individual vessel containing at least 40 ml of Soybean Casein Digest Medium. Additional medium shall be used if the determination required in §113.25(d) indicates the need for a greater dilution of the product. Incubation shall be at 20 °to 25 °C for 14 days.

(6) Examine the contents of all test vessels macroscopically for microbial growth at the end of the incubation period. If growth in a vessel cannot be reliably determined by visual examination, judgment shall be confirmed by subcultures, microscopic examination, or both.

(7) For each set of test vessels representing a serial or subserial tested according to these procedures, the following rules shall apply:

(i) If growth is found in 2 or 3 test vessels of the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final container samples.

(ii) If no growth is found in 9 or 10 of the test vessels in the initial test, or 19 or 20 vessels in the retest, the serial or subserial meets the requirements of the test.

(iii) If growth is found in four or more test vessels in the initial test, or two or more in a retest, the serial or subserial is unsatisfactory.

(b) *Live bacterial vaccines.* Each serial or subserial of live bacterial vaccine shall be tested for purity as prescribed in this paragraph.

(1) Soybean Casein Digest Medium and Fluid Thioglycollate Medium shall be used.

(2) Ten final container samples from each serial and subserial shall be tested.

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and desiccated vaccine shall be rehydrated as recommended on the label with accompanying diluent or with sterile purified water. Product recommended for mass vaccination