AUTHENTICATED
U.S. GOVERNMENT
INFORMATION
GPO

§ 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 shall be rehydrated at the rate of 30 ml sterile purified water per 1,000 doses.

(4) To test for extraneous bacteria, place 0.2 ml of vaccine from each final container into a corresponding individual vessel containing at least 40 ml of Fluid Thioglycollate 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 extraneous fungi, place 0.2 ml of vaccine from each final container 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 atypical microbial growth at the end of the incubation period. If growth of extraneous microorganisms cannot be reliably determined by visual examination, judgment shall be confirmed by subculturing, 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 extraneous 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 extraneous growth is found in 9 or 10 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 extraneous growth is found in 4 or more test vessels in the initial test, or 2 or more in a retest, the serial or subserial is unsatisfactory.

(c) *Master Seed Virus.* Not less than 4 ml of each lot of Master Seed Virus shall be tested. Frozen liquid Master Seed Virus shall be thawed, and desiccated Master Seed Virus shall be rehydrated with Soybean Casein Digest Medium immediately prior to starting the test.

(1) To test for bacteria, place 0.2 ml of the sample of Master Seed Virus into 10 individual vessels each containing at least 120 ml of Soybean Casein Digest Medium. Incubation shall be at 30 °to 35 °C for 14 days.

(2) To test for fungi, place 0.2 ml of the sample of Master Seed Virus into 10 individual vessels each containing at least 40 ml of Soybean Casein Digest Medium. Incubation shall be at 20 °to 25 °C for 14 days.

(3) 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.

(4) For each set of test vessels representing a lot of Master Seed Virus tested according to these procedures, the following rules shall apply:

(i) If growth is found in any test vessel of the initial test, one retest to rule out faulty technique may be conducted using a new sample of Master Seed Virus.

(ii) If growth is found in any test vessel of the final test, the lot of Master Seed Virus is unsatisfactory.

(d) *Master Seed Bacteria.* Not less than 4 ml of each lot of Master Seed Bacteria shall be tested. Frozen liquid Master Seed Bacteria shall be thawed, and desiccated Master Seed Bacteria shall be rehydrated with sterile purified water immediately prior to starting the test.

(1) To test for extraneous bacteria, place 0.2 ml of the sample of Master Seed Bacteria into 10 individual vessels each containing at least 40 ml of Fluid Thioglycollate Medium. Incubation shall be at 30 °to 35 °C for 14 days.

(2) To test for extraneous fungi, place 0.2 ml of the sample of Master Seed Bacteria into 10 individual vessels each containing at least 40 ml of Soybean Casein Digest Medium. Incubation shall be at 20 °to 25 °C for 14 days.

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

(4) For each set of test vessels representing a lot of Master Seed Bacteria tested according to these procedures, the following rules shall apply:

(i) If extraneous growth is found in any test vessel of the initial test, one retest to rule out faulty technique may be conducted using a new sample of Master Seed Bacteria.

(ii) If extraneous growth is found in any test vessel of the final test, the lot of Master Seed Bacteria is unsatisfactory.

(e) Live viral vaccines of chicken embryo origin recommended for administration other than by parenteral injection, which were not tested or have not been found free of bacteria and fungi by the procedures prescribed in paragraph (a) of this section, may be tested according to the procedures prescribed in this paragraph.

(1) Brain Heart Infusion Agar shall be used with 500 Kinetic (Kersey) units of penicillinase per ml of medium added just prior to pouring the plates.

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

(3) Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and lyophilized vaccine shall be rehydrated to the quantity recommended on the label using the accompanying sterile diluent or sterile purified water. Product recommended for mass vaccination shall be rehydrated at the rate of 30 ml sterile purified water per 1,000 doses.

(4) From each container sample, each of 2 plates shall be inoculated with vaccine equal to 10 doses if the vaccine is recommended for poultry or 1 dose if the vaccine is recommended for other animals. Twenty ml of medium shall be added to each plate. One plate shall be incubated at 30 °to 35 °for 7 days and the other plate shall be incubated at 20 °to 25 °C for 14 days.

(5) Colony counts shall be made for each plate at the end of the incubation period. An average colony count for the 10 samples representing the serial or subserial shall be made for each incubation condition.

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

(i) If the average count at either incubation condition exceeds 1 colony per dose for vaccines recommended for poultry, or 10 colonies per dose for vaccines recommended for other animals in the initial test, 1 retest to rule out faulty technique may be conducted using 20 unopened final containers.

(ii) If the average count at either incubation condition of the final test for a serial or subserial exceeds 1 colony per dose for vaccines recommended for poultry, or 10 colonies per dose for vaccines recommended for other animals, the serial or subserial is unsatisfactory.

[48 FR 28430, June 22, 1983, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.28 Detection of mycoplasma contamination.

The heart infusion test, using heart infusion broth and heart infusion agar, provided in this section shall be conducted when a test for mycoplasma contamination is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Media additives provided in this paragraph shall be prepared as follows:

(1) DPN-Cysteine Solution:

(i) Use Nicotinamide adenine dinucleotide (oxidized) and L-Cysteine hydrochloride.

(ii) Prepare 1 gram/100 milliliters (ml) purified water (1 percent solution) of each. Mix the solutions together; the cysteine reduces the DPN. Filter sterilize, dispense in appropriate amounts and store frozen at −20 degrees centigrade.

(2) Inactivated horse serum—horse serum which has been inactivated at 56 °C for 30 minutes.

(b) Heart infusion broth shall be prepared as provided in this paragraph.

(1) Dissolve in 970 ml of purified water, 25 grams of heart infusion broth, 10 grams of proteose peptone No. 3, and either 5 grams of yeast autolysate or 5 ml of fresh yeast extract.

(2) Add the following:

1 percent tetrazolium chloride (ml)	5.5
1 percent thallium acetate (ml)	25
Penicillin (units)	500,000
Inactivated horse serum (ml)	100

(3) Adjust pH to 7.9 with NaOH, filter sterilize, and dispense 100 ml aliquots into 125 ml flasks and store until needed.

(4) Add 2 ml of DPN-Cysteine solution to each 100 ml of broth on day of use.

(c) Heart Infusion Agar shall be prepared as provided in this paragraph.

(1) Dissolve in 900 ml of purified water by boiling the following:

Heart infusion agar (g)	25
Heart–infusion broth (g)	10
Proteose peptone No. 3 (g)	10
1 pct thallium acetate (ml)	25

(2) Cool the medium and adjust pH to 7.9 with NaOH.

(3) Autoclave the medium.

(4) Cool the medium 30 minutes in a 56 °C waterbath.

(5) Dissolve 5 grams of yeast autolysate in 100 ml of distilled water, filter sterilize, and add to the medium.

(6) Add to the medium:

126 ml of inactivated horse serum
21 ml of DPN-Cysteine solution
525,000 units of Penicillin.
Dispense 10 ml aliquots into 60×15 mm disposable culture dishes or petri dishes.

(d) The test procedure provided in this paragraph shall be followed when conducting the mycoplasma detection test.

(1) Preparation of inoculum. Immediately prior to starting the test, frozen liquid vaccine shall be thawed, and lyophilized vaccine shall be rehydrated to the volume recommended on the label with mycoplasma medium. In the case of a lyophilized biological product, e.g., 1,000 dose vial of poultry vaccine to be administered via the drinking