§ 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 °C 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 °C 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.

(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 °C 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 °C 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.

Animal and Plant Health Inspection Service, USDA
§ 113.27

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 °C 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 °C 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 °C 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 °C 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 °C 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 °C 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.
§ 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 percent tetrazolium chloride (ml)</td>
<td>5.5</td>
</tr>
<tr>
<td>1 percent thallium acetate (ml)</td>
<td>25</td>
</tr>
<tr>
<td>Penicillin (units)</td>
<td>500,000</td>
</tr>
<tr>
<td>Inactivated horse serum (ml)</td>
<td>100</td>
</tr>
</tbody>
</table>

(3) Adjust pH to 7.9 with NaOH, filter sterilize, and dispense 100 ml aliquots.