of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[43 FR 25077, June 9, 1978, as amended at 48 FR 31008, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.123 Salmonella Dublin Bacterin.

Salmonella Dublin Bacterin shall be prepared from a culture of *Salmonella dublin* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella dublin* fraction shall meet the applicable requirements in 9 CFR 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in 9 CFR 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Veterinary Services.

(2) At least three tenfold dilutions shall be made with the Standard and the same tenfold dilutions shall be made for each Unknown. The dilutions shall be made in Phosphate-Buffered Saline.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 vaccinated mice per group shall be challenged intraperitoneally 7 to 10 days after the second vaccination with a 0.25 ml dose containing 1,000–100,000 mouse LD_{50} as determined by titration of a suitable culture of *Salmonella dublin*. All survivors in each group of mice shall be recorded 14 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.30, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test; *Provided*, That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[43 FR 25077, June 9, 1978, as amended at 48 FR 31009, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

KILLED VIRUS VACCINES

§ 113.200 General requirements for killed virus vaccines.

When prescribed in an applicable Standard Requirement or in the filed Outline of Production, a killed virus vaccine shall meet the applicable requirements in this section.

(a) *Killing agent.* The vaccine virus shall be killed (inactivated) by an appropriate agent. The procedure involved may be referred to as inactivation. Suitable tests to assure complete inactivation shall be written into the filed Outline of Production.

(b) *Cell culture requirements.* If cell cultures are used in the preparation of the vaccine, primary cells shall meet the requirements in § 113.51 and cell lines shall meet the requirements in § 113.52.

(c) *Purity tests*—(1) *Bacteria and fungi.* Final container samples of completed product from each serial shall be tested as prescribed in § 113.26.

(2) *Avian origin vaccine.* Bulk pooled material or final container samples from each serial shall also be tested for:

(i) *Salmonella* contamination as prescribed in § 113.30; and

(ii) *Lymphoid leukosis virus* contamination as prescribed in § 113.31; and

(iii) *Hemagglutinating viruses* as prescribed in § 113.34.

(3) *Mycoplasma.* If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for mycoplasma as prescribed in § 113.28, prior to adding the killing agent. Material found to contain mycoplasma is unsatisfactory for use.

(4) *Extraneous viruses.* Each lot of Master Seed Virus used to prepare killed virus vaccine recommended for animals other than poultry shall meet the requirements for extraneous viruses as prescribed in § 113.55.