

§ 113.313

9 CFR Ch. I (1-1-07 Edition)

titer equal to or greater than that used in the immunogenicity test.

(3) Young adult mice, each weighing 14 to 16 grams, shall be used as test animals when the virus in vaccine prepared with a low egg passage Flury Strain or high cell passage Street Alabama Dufferin Strain (HCP SAD) of rabies virus is titrated. At least 10 mice for each dilution shall be used.

(i) At least 10 mice shall be used for each dilution. Each shall be injected intracerebrally with 0.03 ml.

(ii) The injected young adult mice shall be observed each day for 14 days except when testing vaccines made with HCP SAD strain of rabies virus, in which case, the mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD<sub>50</sub> titer calculated by the Reed and Muench Method.

(iii) Virus titer requirements for release and at expiration date shall be determined for each vaccine on the basis of data available: *Provided*, That, the lowest titer permitted at expiration date when determined by this test shall be 10<sup>3.0</sup> LD<sub>50</sub> per 0.03 ml.

(4) Suckling mice, 6 days of age or younger, shall be used as test animals when virus in vaccine prepared with a high egg passage Flury Strain of rabies virus is titrated.

(i) Six to twelve mice shall be used for each dilution. Each shall be injected intracerebrally with 0.02 ml.

(ii) The injected suckling mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD<sub>50</sub> titer calculated by the Reed and Muench Method; and

(iii) Virus titer requirements for release and at expiration date shall be determined for each vaccine on the basis of data available: *Provided*, That, the lowest titer permitted at expiration date when determined by this test shall be 10<sup>3.0</sup> LD<sub>50</sub> per 0.02 ml.

[39 FR 44721, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 42 FR 6795, Feb. 4, 1977; 43 FR 49529, Oct. 24, 1978; 50 FR 20090, May 14, 1985; 50 FR 23797, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 61 FR 31823, June 21, 1996]

§ 113.313 Measles Vaccine.

Measles Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300. Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(b) To detect virulent canine distemper virus, each of two canine distemper susceptible ferrets shall be injected with a sample of the Master Seed Virus equivalent to the amount of virus to be used in one dog dose and observed each day for 21 days. If undesirable reactions occur in either ferret, the lot of Master Seed Virus is unsatisfactory.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five dogs, less than 12 weeks of age and free of measles antibody, shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 ID<sub>50</sub> of measles virus.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Twenty dogs shall be vaccinated with a predetermined quantity of vaccine virus and the remaining five dogs held as unvaccinated controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) On the day of challenge, serum samples shall be obtained from each vaccinate and individually tested for antibody against canine distemper virus. For a valid test, each vaccinate shall be negative at a 1:4 final serum dilution in varying serum-constant virus neutralization test using less than 500 ID<sub>50</sub> of canine distemper virus.

(4) At least 21 days postinoculation, the immunity of the vaccinates and controls shall be challenged by exposure to a uniform dose of aerosolized virulent canine distemper virus. All test dogs shall be observed daily for 21 days postchallenge.

(i) If at least 4 of the 5 controls do not die or show signs of distemper, including a temperature of 104.0 °F. or higher and at least 15 percent weight loss, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive without showing a temperature of 104.0 °F. or higher and a weight loss exceeding 15 percent after day 8 postchallenge, the Master Seed Virus is unsatisfactory.

(5) When approved in advance by Animal and Plant Health Inspection Service, a sequential test procedure may be used in lieu of the 20 dog requirement. A beta value of 0.05 and a tolerance level of 0.78 shall be required.

(6) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided*, That five of five vaccinates and at least four of the controls shall meet the criteria prescribed in this section.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety tests.* The dog safety test prescribed in §113.40 and the mouse

safety test prescribed in §113.33(a) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of the vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of 10<sup>0.7</sup> greater than that used in the immunogenicity test but not less than 10<sup>2.5</sup> ID<sub>50</sub> per dose.

[40 FR 53001, Nov. 14, 1975, as amended at 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

#### § 113.314 Feline Calicivirus Vaccine.

Feline Calicivirus Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(b) The Master Seed Virus shall be tested for chlamydial agents as prescribed in §113.43.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Thirty feline calicivirus susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline calicivirus. Blood samples shall be drawn and individual serum samples tested. The cats shall be considered