

administered, five replicate virus titrations shall be conducted on a sample of the vaccine used.

(3) At least once during the period of 14 to 18 days postvaccination, individual serum samples shall be collected from each of the vaccinates and tested for virus neutralizing antibody using the 60 to 300 TCID<sub>50</sub> of bluetongue virus.

(4) Twenty-one to twenty-eight days postvaccination the vaccinates and the controls shall each be challenged with virulent bluetongue virus and observed for 14 days. The rectal temperature of each animal shall be taken and recorded for 17 consecutive days beginning 3 days prechallenge. The presence or absence of lesions or other clinical signs of bluetongue noted and recorded on each of 14 consecutive days postchallenge.

(i) If at least four of the five controls do not show clinical signs of bluetongue and a temperature rise of 3 ° F or higher over the prechallenge mean temperature, the test shall be considered inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates tested as prescribed in paragraph (c)(3) of this section do not have bluetongue neutralizing antibody titers of 1:4 final serum dilution or higher, or if more than one of the vaccinates shows a temperature rise of 3 ° F or higher than its prechallenge mean temperature for 2 or more days, or if more than one of the vaccinates exhibits clinical signs of bluetongue, the Master Seed is unsatisfactory.

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

(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 be used in the retest: *Provided*, That five of five vaccinates and at least four of the five controls shall meet the criteria prescribed in paragraphs (c)(4) of this section.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements pre-

scribed 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 test.* The mouse safety test prescribed in §113.33(a) and the lamb safety test prescribed in §113.45 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 subserial shall have a virus titer sufficiently greater than the titer of 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 such immunogenicity test.

[50 FR 23796, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

#### § 113.304 Feline Panleukopenia Vaccine.

Feline Panleukopenia 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 and the requirements prescribed in this section.

(b) The lot of Master Seed Virus shall be tested for other agents as follows:

(1) To detect virulent feline panleukopenia virus or virulent mink enteritis virus, each of two feline panleukopenia susceptible cats, as determined by the criteria prescribed in paragraph (c)(1) of this section, shall be injected subcutaneously with the equivalent of one cat dose each and the cats observed each day for 21 days. If either or both cats show signs of disease or reduced white blood cell counts

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below 50 percent of the normal level established by an average of three or more counts taken prior to injection, the Master Seed Virus is unsatisfactory.

(2) To detect chlamydial agents, the Master Seed Virus shall be tested 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) Twenty-five feline panleukopenia susceptible cats shall be used as test animals (20 vaccinates and 5 controls). Blood samples drawn from each cat shall be individually tested for neutralizing antibody against feline panleukopenia virus to determine susceptibility.

(i) A constant virus-carrying serum neutralization test in tissue culture using 100 to 300 TCID<sub>50</sub> of virus shall be used.

(ii) Cats shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(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. The 20 cats used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five cats held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) Fourteen days post-injection, the vaccinates and the controls shall be challenged with virulent feline panleukopenia virus furnished by Animal and Plant Health Inspection Service and the cats observed each day for 14 days.

(i) If at least 80 percent of the controls do not show clinical signs of feline panleukopenia during the observation period, the test is inconclusive and may be repeated. Clinical signs of feline panleukopenia shall include a pronounced leukopenia wherein the white cell count drops to 4,000 or less per cubic mm, or the white cell count drops to less than 25 percent of the nor-

mal level established by an average of three or more counts taken prior to challenge.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing clinical signs of feline panleukopenia as described in paragraph (c)(3)(i) of this section, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Ten susceptible cats (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (c)(1) of this section.

(i) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (c)(2) of this section.

(ii) Fourteen to twenty-one days postvaccination, a second serum sample shall be drawn from each cat and tested for neutralizing antibody to feline panleukopenia virus in the same manner used to determine susceptibility.

(iii) If the two controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If at least 6 of the 8 vaccinates in a valid test do not develop titers based upon final serum dilution of at least 1:8, and the remaining vaccinates do not develop titers of at least 1:4, the Master Seed Virus is unsatisfactory except as provided in paragraph (c)(4)(v) of this section.

(v) If the results of a valid SN test are unsatisfactory, the vaccinates and the controls may be challenged as provided in paragraph (c)(3) of this section. If 100 percent of the controls do not show clinical signs of feline panleukopenia, the test is inconclusive and may be repeated except, that, if any of the vaccinates show such signs, the Master Seed Virus is unsatisfactory.

(5) 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 requirements prescribed in § 113.304 and

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 Test.* The mouse safety test prescribed in §113.33(a) and the cat safety test prescribed in §113.39 shall be conducted.

(i) Each of two healthy cats shall be injected with 10 cat doses by the method recommended on the label and the cats observed each day for 14 days.

(ii) If unfavorable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided*, That, if not repeated, the serial shall be unsatisfactory.

(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 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  $10^{0.7}$  greater than that used in such immunogenicity test but not less than  $10^{2.5}$  TCID<sub>50</sub> per dose.

[39 FR 44716, Dec. 27, 1974, as amended at 40 FR 53378, Nov. 18, 1975; 43 FR 25078, June 9, 1978; 43 FR 41186, Sept. 15, 1978; 44 FR 58900, Oct. 12, 1979; 48 FR 33471, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

#### § 113.305 Canine Hepatitis and Canine Adenovirus Type 2 Vaccine.

Canine Hepatitis Vaccine and Canine Adenovirus Type 2 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 in preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 except that the dog safety test prescribed in §113.40(a) shall be conducted by the intravenous route.

(b) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity by one or both of the following methods:

(1) *Immunogenicity for canine hepatitis.* Twenty-five canine hepatitis susceptible dogs shall be used as test animals (20 vaccinates and 5 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 using 50 to 300 TCID<sub>50</sub> of canine adenovirus.

(i) 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. The 20 dogs to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five dogs held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(ii) Not less than 14 days postinjection, the vaccinates and the controls shall each be challenged intravenously with virulent infectious canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days.

(A) If at least four of the five controls do not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated.

(B) If at least 19 of the 20 vaccinates do not survive without showing clinical signs of infectious canine hepatitis during the observation period, the Master Seed Virus is unsatisfactory.

(iii) The Master Seed Virus shall be retested for immunogenicity for canine hepatitis in 3 years unless use of the lot previously tested is discontinued. Ten susceptible dogs (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in