

(v) *Virus challenge test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the control(s) may be challenged intravenously with a virulent canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and each animal observed each day for an additional 14 days.

(vi) *Interpretation of the virus challenge test.* For a serial to be satisfactory, all vaccinates must remain free of clinical signs of canine hepatitis while the control(s) must show severe clinical signs of canine hepatitis. If the control(s) does not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated: *Provided*, That, if any of the vaccinates show signs or die of canine hepatitis, the serial is unsatisfactory.

(3) *Potency test for canine adenovirus type 2.* Bulk or final container samples of completed product shall be tested for potency using eight susceptible dogs (five vaccinates and three controls) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine adenovirus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 TCID<sub>50</sub> of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination.* Each of the five vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) Not less than 14 days after the last inoculation, the vaccinates and the controls shall be challenged by exposure to a nebulized aerosol of virulent canine adenovirus type 2 furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence of respiratory or other clinical signs of canine adenovirus type 2 noted and recorded each day.

(iv) If at least two of three controls do not show clinical signs of canine adenovirus type 2 other than fever, the test is inconclusive and may be repeated.

(v) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the serial is unsatisfactory.

[60 FR 14359, Mar. 17, 1995]

#### § 113.203 Feline Panleukopenia Vaccine, Killed Virus.

Feline Panleukopenia Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed. The Master Seed shall meet the applicable requirements prescribed in § 113.200. Each serial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements for safety and potency provided in this section.

(a) *Safety test.* The vaccinates used in the potency test in paragraph (b) of this section shall be observed each day during the postvaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided*, That, if not repeated, the serial is unsatisfactory.

(b) *Potency test—serum-neutralization test.* Bulk or final container samples of completed product shall be tested for potency using five susceptible cats (four vaccinates and one control) as the test animals. Blood samples drawn from each cat shall be individually tested for neutralizing antibody against feline panleukopenia virus to determine susceptibility.

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

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

(2) *Vaccination.* Each of the four vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be given 7 to 10 days after the first dose and the cats observed each day for 14 to 21 days.

(3) *Serology.* At the end of the postvaccination observation period, a second blood sample shall be obtained from each of the five cats and the serums shall be individually tested for neutralizing antibody against feline panleukopenia virus in the same manner used to determine susceptibility.

(4) *Interpretation of the SN test.* (i) If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(ii) If at least 3 of the 4 vaccinates in a valid test have not developed titers based upon final serum dilution of at least 1:8, and the remaining vaccinate has not developed a titer of at least 1:4, the serial is unsatisfactory except as provided in paragraphs (b)(5) and (6) of this section.

(5) *Virus-challenge test.* If the results of a valid SN test are unsatisfactory, the vaccinates and the control may be challenged with a virulent feline panleukopenia virus furnished by Veterinary Services and each animal observed each day for an additional 14 days.

(6) *Interpretation of the virus-challenge test.* If the control does 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 serial is unsatisfactory. Clinical signs of feline panleukopenia shall include a pronounced leukopenia wherein the white blood cell count drops to 4,000 or less per cubic mm or the white cell count drops to less than 25 percent of the normal level established by an average of three or more counts taken prior to challenge.

[39 FR 27428, July 29, 1974, as amended at 40 FR 759, Jan. 3, 1975; 43 FR 41186, Sept. 15, 1978; 43 FR 50162, Oct. 27, 1978; 50 FR 23796, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

#### § 113.204 Mink Enteritis Vaccine, Killed Virus.

Mink Enteritis Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids or tissues obtained from mink that have developed mink enteritis following inoculation with virulent mink enteritis virus. Each serial shall meet the applicable requirements prescribed in § 113.200 and special requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety test.* Vaccinates used in the potency test in paragraph (b) of this section shall be observed each day prior to challenge. If unfavorable reactions attributable to the vaccine occur, the serial is unsatisfactory. If unfavorable reactions not attributable to the vaccine occur, the test shall be declared inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial is unsatisfactory.

(b) *Potency test.* Bulk or final container samples of completed product shall be tested for potency using 10 mink enteritis susceptible mink (five vaccinates and five controls) as follows:

(1) *Vaccination.* Each of the five vaccinates shall be injected with one dose of vaccine as recommended on the label and observed each day for 14 days.

(2) *Challenge.* At least 2 weeks after the last inoculation, the five vaccinates and the five controls shall be challenged with virulent mink enteritis virus and observed each day for 12 days. Fecal material shall be collected on one day between days 4-8 (inclusive) postchallenge from each test animal that remains free of enteric signs and tested for the presence of mink enteritis virus by cell culture with fluorescent antibody examination.

(3) *Interpretation.* A serial is satisfactory if at least 80 percent of the vaccinates remain free of enteric signs and do not shed virus in the feces, while at least 80 percent of the controls develop clinical signs of mink enteritis or shed virus in the feces. If at least 80 percent of the vaccinates remain free of enteric signs and do not shed virus in the feces, while less than 80 percent of the controls develop clinical signs of mink enteritis or shed virus in the feces, the