section shall be observed each day during the prechallenge period. If unfavorable reactions occur, including neurological signs, 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. If the test is not repeated, the serial is unsatisfactory.

(2) *Potency*. Bulk or final container samples of completed product shall be tested for potency as follows:

(i) Ten pseudorabies susceptible pigs (five vaccinates and five controls) shall be used as test animals. The animals shall be at the minimal age recommended for vaccination. Blood samples shall be drawn and individual serum samples inactivated and tested for neutralizing antibody.

(ii) A constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of virus shall be used. Pigs shall be considered susceptible if there is no neutralization at 1:2 final serum dilution. Other tests of equal sensitivity acceptable to Animal and Plant Health Inspection Service may be used.

(iii) The five pigs used as vaccinates shall be administered one dose of vaccine as recommended on the label. If two doses are recommended, the second dose shall be given after the interval recommended on the label.

(iv) Fourteen days or more after vaccination, blood samples shall be drawn and individual serum samples inactivated and tested for pseudorabies virus neutralizing antibody by the method used to determine susceptibility.

(v) *Test interpretation*. If the controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at least four of the five vaccinates in a valid test have not developed titers 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 paragraph (c)(2)(vi) of this section.

(vi) *Virus challenge test*. If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and controls may be challenged with virulent pseudorabies virus furnished or approved by Animal and Plant Health Inspection Service. The animals shall be observed each day for 14 days postchallenge. If four of five controls do not develop central nervous system signs or die, the test is inconclusive and may be repeated. In a valid test, if two or more of the vaccinates develop clinical signs or die, the serial is unsatisfactory.

[50 FR 434, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.214 Parvovirus Vaccine, Killed Virus (Canine).

Parvovirus Vaccine, Killed Virus, recommended for use in dogs, 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 vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared in accordance with the Outline of Production shall be established as follows:

(1) Twenty-five parvovirus susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine parvovirus to determine susceptibility.

A constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution. Other tests of equal sensitivity acceptable to Animal and Plant Health Inspection Service may be used.

(2) A viral hemagglutination test or another test acceptable to Animal and Plant Health Inspection Service shall be used to measure the antigenic content of vaccine produced at the highest passage from the Master Seed before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be injected with a predetermined dose of vaccine by the method recommended on the label. To confirm the dosage calculations, five replicate tests shall be

conducted on a sample of the vaccine used. If two doses are used, five replicate confirming tests shall be conducted on each dose.

(3) Fourteen days or more after the final dose of vaccine, the vaccinates and the controls shall be challenged with virulent canine parvovirus furnished or approved by Animal and Plant Health Inspection Service and the dogs observed each day for 14 days. Rectal temperature, blood lymphocyte count, and feces for viral detection shall be taken from each dog each day for at least 10 days postchallenge and the presence or absence of clinical signs noted and recorded each day.

(i) The immunogenicity of the vaccine shall be evaluated on the following criteria of infection: temperature ≥103.4 ° F; lymphopenia of ≥50 percent of prechallenge normal; clinical signs such as diarrhea, mucus in feces, or blood in feces; and viral hemagglutinins at a level of ≥1:64 in a 1:5 dilution of feces or a test of equal sensitivity. If at least 80 percent of the controls do not show at least three of the four criteria of infection during the observation period, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing any more than one criterion of infection described in subparagraph (3)(i), of this section, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five susceptible dogs (four vaccinates and one control) need to be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (b)(1) of this section.

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

(ii) Fourteen to 21 days after the last vaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine parvovirus in the same manner used to determine susceptibility.

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

(iv) If three of the four vaccinates in a valid test do not develop titers based upon final serum dilution of at least 1:16, and the remaining vaccinate does not develop a titer of at least 1:8, the Master Seed is unsatisfactory, except as provided in subparagraph (4)(v) of this section.

(v) If the results of a valid SN test are unsatisfactory, the vaccinates and the control may be challenged as provided in paragraph (b)(3) of this section. If at least three of the four criteria of infection are not shown, the test is inconclusive and may be repeated, except that if any of the vaccinates show more than one criterion of infection, 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.

(c) *Test requirements for release*. Each serial and subserial shall meet the requirements prescribed in §113.200 and in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Potency*. Bulk or final container samples of completed product shall be tested for antigenic content using the method used in paragraph (b)(2) of this section. To be eligible for release, each serial and each subserial shall have an antigenic content sufficiently greater than that used in the immunogenicity test to assure that, when tested at any time within the expiration period, each serial and subserial shall have an antigenic content equal to the amount used in such immunogenicity test.

(2) *Virus identity*. Bulk or final container samples shall be tested for virus identity by conducting a hemagglutination test using duplicate samples and pretreating one with specific canine parvovirus antibody. If there is not at least an eightfold reduction in hemagglutinating activity, the hemagglutination is considered to be nonspecific and the serial is unsatisfactory.

[50 FR 435, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]