(iii) *Challenge.* Twenty-one to 28 days postvaccination, challenge 20 vaccinates and 10 controls by eyedrop with a virulent infectious bursal disease virus furnished or approved by Animal and Plant Health Inspection Service.

(iv) *Postchallenge period.* Four days postchallenge, necropsy all chickens and examine each for gross lesions of bursal disease. For purposes of this test, gross lesions shall include peribursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue. Vaccinated chickens showing gross lesions shall be counted as failures. If at least 80 percent of the controls do not have gross lesions of bursal disease in a stage of the test, that stage is considered inconclusive and may be repeated. In a valid test, the results shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of failures for—	
			Satisfactory serial	Unsatisfactory serial
1	20	20	3 or less	6 or more.
2	20	40	8 or less	9 or more.

(v) If four or five vaccinates show lesions of bursal disease in the first stage, the second stage may be conducted in a manner identical to the first stage. If the second stage is not conducted, the serial is unsatisfactory.

(vi) If the second stage is used, each serial shall be evaluated according to the second part of the table on the basis of cumulative results.

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

§ 113.213 Pseudorabies Vaccine, Killed Virus.

Pseudorabies 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.

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

(b) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production shall be established by a method acceptable to Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production.

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

(1) *Safety.* Vaccinates used in the potency test in paragraph (c)(2) of this 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