

immunogenicity test, but not less than $10^{2.5}$ ID₅₀ per dose.

[50 FR 436, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§ 113.318 Pseudorabies Vaccine.

Pseudorabies Vaccine 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 §113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five pseudorabies susceptible pigs (20 vaccinates and 5 controls) of the youngest age for which the vaccine is recommended, shall be used as test animals. Blood samples shall be taken from each pig and the serums inactivated and individually tested for neutralizing antibody against pseudorabies virus. Pigs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test using 50 to 300 TCID₅₀ pseudorabies virus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 pigs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) Fourteen to 28 days postvaccination, the vaccinates and controls shall be challenged with virulent pseudorabies virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days.

(i) If at least four of the five controls do not develop severe central nervous system signs or die, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates in a valid test do not remain free of signs of pseudorabies, the Master Seed is unsatisfactory.

(4) 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 applicable general requirements prescribed in §113.300 and the requirements in this paragraph.

(2) *Virus titer requirements.* Final container samples of completed product shall be titrated by the method used in paragraph (b)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer at least $10^{0.7}$ greater than that used in the immunogenicity test, but not less than $10^{2.5}$ TCID₅₀ per dose.

[50 FR 437, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§§ 113.319–113.324 [Reserved]

§ 113.325 Avian Encephalomyelitis Vaccine.

Avian Encephalomyelitis Vaccine shall be prepared from virus-bearing tissues or fluids from embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for 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 and the requirements prescribed in this section.

§ 113.325

9 CFR Ch. I (1–1–11 Edition)

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Avian encephalomyelitis susceptible chickens, all of the same age (eight weeks or older) and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed ten-fold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inoculate at least 10 embryos, 5 or 6 days old, in the yolk sac with 0.2 ml each. Twenty similar embryos obtained from the same source shall be kept as uninoculated negative controls. Disregard all deaths during the first 48 hours post-inoculation.

(ii) Eggs for each dilution shall be kept in separate containers and allowed to hatch. Sufficient precaution shall be taken to assure that chickens from each dilution remain separated. To be a valid test, at least 75 percent of the uninoculated eggs shall hatch.

(iii) On the third day after normal hatching time, count all unhatched eggs and all dead, paralyzed and ataxic chickens as positive evidence of viral infection.

(iv) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(v) Calculate the EID_{50} by the Spearman-Kärber or Reed-Muench method.

(3) At least 21 days post-vaccination, the vaccinates and the controls shall be challenged intracerebrally with a virulent avian encephalomyelitis virus and observed each day for 21 days.

(4) If at least 80 percent of the controls do not show signs of avian encephalomyelitis or die, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of avian encephalomyelitis during the observation period, 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) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300 and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the vaccine judged accordingly.

(2) *Safety test.* Final container samples of completed product shall be tested for safety as follows:

(i) At least 25 AE susceptible birds (6 to 10 weeks of age) shall be vaccinated with the equivalent of 10 doses by each of all routes recommended on the label and be observed each day for 21 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, except that, if the test is not repeated, the serial shall be unsatisfactory.

(3) *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 $10^{0.5}$ EID₅₀ per dose.

[39 FR 44723, Dec. 27, 1974, as amended at 40 FR 18405, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§ 113.326 Avian Pox Vaccine.

Fowl Pox Vaccine and Pigeon Pox Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for 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 paragraph (c) of this section and shall meet the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken inoculation test prescribed in § 113.36.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Fowl pox susceptible birds all of the same age and from the same source, shall be used as test birds. Twenty or more birds shall be used as vaccinates for each method of administration recommended on the label. Ten

additional birds of the same age and from the same source as the vaccinates shall be held as unvaccinated controls.

(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. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each bird used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inoculate at least five embryos, 9 to 11 days old, on the chorioallantoic membrane with at least 0.2 ml each. Disregard all deaths during the first 24 hours post-inoculation. To be a valid test, at least four embryos in each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 7 days post-inoculation.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID₅₀ by the Spearman-Kärber or Reed-Muench method.

(3) Fourteen to twenty-one days post-vaccination, all vaccinates and controls shall be challenged by the wing web method and observed each day for 10 days. If the wing web method was used for vaccination, the opposite wing shall be used for challenge. Challenge virus shall be provided or approved by Animal and Plant Health Inspection Service.

(4) If at least 90 percent of the controls do not develop fowl pox during the observation period, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of fowl pox during the observation period, 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