§ 113.207 Encephalomyelitis Vaccine, Eastern, Western, and Venezuelan, Killed Virus.

Encephalomyelitis Vaccine, Eastern, Western, and Venezuelan, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Each serial or subserial shall meet the requirements prescribed in this section and the general requirements prescribed in § 113.200, except those in § 113.200(d). Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety test.* Bulk samples of completed product from each serial shall be tested for encephalomyelitis virus inactivation.

(1) Each of at least ten 6 to 12 hour old chickens shall be injected subcutaneously with 0.5 ml of the product and the chickens observed each day for 10 days.

(2) If unfavorable reactions attributable to the product occur in the chickens during the observation period, the serial is unsatisfactory. If unfavorable reactions not attributable to the product occur, the test is 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 from each serial shall be tested for potency in accordance with the two-stage test provided in this paragraph. For each fraction contained in the product—Eastern type, Western type, or Venezuelan type—the serological interpretations required in this test shall be made independently. A serial or subserial found unsatisfactory for any of the fractions shall not be released.

(1) For this test, a guinea pig dose shall be one-half the amount recommended on the label for a horse and shall be administered as recommended for a horse. Each of 10 healthy guinea pigs (vaccinates) shall be injected with two guinea pig doses with an interval of 14 to 21 days between doses. Two additional guinea pigs from the same source shall be held as controls.

(2) Fourteen to 21 days after the second injection, serum samples from each vaccinate and each control shall be tested by a plaque reduction, serum neutralization test using Vero 76 cells.

(3) If the control serum samples show a titer of 1:4 or greater for any fraction, the test is inconclusive for that fraction and may be repeated: *Provided*, That, if four or more of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction, less than 1:40 for the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the serial or subserial is unsatisfactory without further testing.

(4) If two or three of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction, less than 1:40 for the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the second stage of the test may be used for the relevant fraction(s): *Provided*, That, if a fraction is found acceptable by the first stage of the test, the second stage need not be conducted for that fraction.

(5) If the second stage is used and four or more of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction or the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the serial or subserial is unsatisfactory.

(6) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Vaccinates	Failures for acceptance	Failures for rejection
1	10	1 or less	4 or more.
2	20	3 or less	Do.

[39 FR 44714, Dec. 27, 1974, as amended at 40 FR 14084, Mar. 28, 1975; 42 FR 45284, Sept. 9, 1977. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991; 61 FR 67930, Dec. 26, 1996]

§ 113.208 Avian Encephalomyelitis Vaccine, Killed Virus.

Avian Encephalomyelitis Vaccine (Killed Virus) shall be prepared from virus-bearing tissues or fluids obtained from embryonated chicken eggs. Each serial shall meet the general requirements prescribed in § 113.200 and the requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety tests.* (1) The prechallenge part of the potency test prescribed in

paragraph (b) of this section shall constitute a safety test. If any of the vaccinates develop clinical signs of disease or die due to causes attributable to the product, the serial is unsatisfactory.

(2) An inactivation test for viable avian encephalomyelitis (AE) virus shall be conducted on each serial. The test shall be conducted using susceptible chicken embryos: *Provided*, That, if a non-embryo adapted virus is used for vaccine production, the test shall be conducted in susceptible chickens.

(i) *Chicken Embryo Test.* Each of 15 or more AE susceptible 5 or 6 day old embryos shall be injected in the yolk sac with 0.2 ml of the vaccine. For a valid test, at least 80 percent of the embryos shall survive for 48 hours post-inoculation (PI). Eleven to 13 days PI, all embryos surviving the 48 hour PI period shall be examined for gross lesions of AE; all these embryos shall be normal or the serial is unsatisfactory. Concurrently, five additional embryos from the same source shall be injected with live AE virus of the production strain to serve as positive controls. At least 4 of the 5 embryos shall show evidence of AE virus infection during the 11 to 13 day PI period or the test shall be considered inconclusive and repeated: *Provided*, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) *Chicken test.* Each of 10 or more AE susceptible 7 day old chickens shall be injected intracerebrally with 0.1 ml vaccine each. The chickens shall be observed each day for 28 days. If any chickens show clinical signs of AE, the serial is unsatisfactory. Concurrently, 5 additional chickens from the same source shall be injected intracerebrally with live AE virus of the production strain to serve as positive controls. At least 4 of the 5 controls shall show evidence of AE virus infection during the observation period or the test shall be inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial shall be unsatisfactory.

(b) *Potency test.* Bulk or final container samples of completed product from each serial or one subserial shall be tested. Ten or more AE-susceptible chickens (vaccinates), 4 weeks or older, properly identified and obtained from the same source and hatch, shall be injected as recommended on the label. At least 10 additional AE-susceptible chickens, properly identified and obtained from the same source and hatch shall be kept in isolation as controls.

(1) At least 28 days post-injection, the vaccinates and the controls shall be challenged intramuscularly with a virulent AE virus and the chickens observed each day for 21 days.

(2) If at least 80 percent of the controls do not show clinical signs of or die from AE infection, the test is inconclusive and may be repeated.

(3) If at least 80 percent of the vaccinates do not remain normal, the serial is unsatisfactory.

[39 FR 12958, Dec. 27, 1974, as amended at 40 FR 41088, Sept. 5, 1975. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.209 Rabies Vaccine, Killed Virus.

Rabies Vaccine (Killed Virus) shall be prepared from virus-bearing cell cultures or nerve tissues obtained from animals that have developed rabies infection following injection with rabies virus. 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 requirements prescribed in § 113.200 and the requirements prescribed in this section.

(1) Each lot of Master Seed Virus propagated in tissue or cells of avian origin shall also be tested for extraneous pathogens by procedures prescribed in § 113.37.

(2) Each lot of Master Seed Virus propagated in primary cell cultures of mouse or hamster origin or brain tissues of mouse origin shall be tested for lymphocytic choriomeningitis (LCM) virus by the procedure prescribed in § 113.42. If LCM virus is detected, the Master Seed Virus is unsatisfactory.

(b) The immunogenicity of vaccine prepared with virus at the highest passage from the Master Seed shall be established in each species for which the vaccine is recommended. Tests shall be