

§ 113.36

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vaccines which are prepared by the licensee and which are licensed. *Provided*, That the Administrator may authorize licensees to prepare and use unlicensed single-fraction vaccines for this purpose.

(c) Test procedure: (1) Rehydrate at least two vials of the vaccine with the liquid product under test according to label recommendations and pool the contents.

(2) Rehydrate at least two vials of the vaccine with the same volume of sterile purified water and pool the contents.

(3) Neutralize to remove other fractions, if necessary.

(4) Hold the two pools of vaccine at room temperature (20 °to 25 °C) for 2 hours. The holding period shall begin when rehydration is completed.

(5) Titrate the virus(es) in each pool of vaccine as provided in the filed Outline of Production or an applicable standard requirement.

(6) Compare respective titers.

(d) If the titer of the vaccine virus(es) rehydrated with the product under test is more than 0.7 log₁₀ below the titer of the vaccine virus(es) rehydrated with sterile purified water, the product is unsatisfactory for use as diluent.

(e) If the product is unsatisfactory in the first test, one retest to rule out faulty techniques may be conducted using four vials of the vaccine for each pool and the acceptability of the product judged by the results of the second test.

(f) Liquid products found to be unsatisfactory for use as diluent by this test are not prohibited from release as separate licensed products if labeled as prescribed in §112.7(g).

[44 FR 25412, May 1, 1979, as amended at 56 FR 66784, Dec. 26, 1991; 64 FR 43044, Aug. 9, 1999]

§ 113.36 Detection of pathogens by the chicken inoculation test.

The test for detection of extraneous pathogens provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) The biological product to be tested shall be prepared for use as recommended on the label, or in the case

of desiccated vaccine to be used in poultry, rehydrated with sterile distilled water at the rate of 30 ml per 1,000 doses.

(b) At least 25 healthy susceptible young chickens, properly identified and obtained from the same source and hatch, shall be immunized at least 14 days prior to being put on test. The immunizing agent shall be the same as the product to be tested but from a serial previously tested and found satisfactory.

(c) At least 20 of the previously immunized birds shall be inoculated with 10 label doses of the vaccine being tested by each of the following routes: Subcutaneous, intratracheal, eye-drop, and comb scarification (1 cm²). Twenty birds may be used for each route or combination of routes.

(d) At least five birds shall be isolated as control birds.

(e) All birds shall be observed for 21 days for signs of septicemic diseases, respiratory diseases, or other pathologic conditions.

(f) If the controls remain healthy and unfavorable reactions attributable to the product occur in the vaccinates, the serial or subserial tested is unsatisfactory. If the controls do not remain healthy or if unfavorable reactions not attributable to the product occur in the vaccinates, or both, the test shall be declared inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial of subserial tested shall be considered unsatisfactory.

[38 FR 29889, Oct. 30, 1973, as amended at 39 FR 21042, June 18, 1974; 43 FR 7610, Feb. 24, 1978]

§ 113.37 Detection of pathogens by the chicken embryo inoculation test.

The test for detection of extraneous pathogens provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) The biological product to be tested shall be prepared for use as recommended on the label, or in the case of desiccated vaccine to be used in poultry, rehydrated with sterile distilled water at the rate of 30 ml per 1,000 doses.

(b) One volume of the prepared vaccine shall be mixed with up to nine volumes of sterile heat-inactivated specific antiserum to neutralize the vaccine virus in the product. Each lot of antiserum shall be demonstrated by virus neutralization tests not to inhibit other viruses known to be possible contaminants.

(c) After neutralization, 0.2 ml of the vaccine-serum mixture shall be inoculated into each of at least 20 fully susceptible chicken embryos.

(1) Twenty embryos, 9 to 11 days old, shall be inoculated on the chorio-allantoic membrane (CAM) with 0.1 ml, and in the allantoic sac with 0.1 ml.

(2) Eggs shall be candled daily for 7 days. Deaths occurring during the first 24 hours shall be disregarded but at least 18 viable embryos shall survive 24 hours post-inoculation for a valid test. Examine all embryos and CAM's from embryos which die after the first day. When necessary, embryo subcultures shall be made to determine the cause of a death. The test shall be concluded on the seventh day post-inoculation and the surviving embryos (including CAM's) examined.

(d) If death and/or abnormality attributable to the inoculum occur, the serial is unsatisfactory: *Provided*, That, if there is a vaccine virus override, the test may be repeated, using a higher titered antiserum.

[38 FR 29889, Oct. 30, 1973, as amended at 39 FR 21042, June 18, 1974]

§ 113.38 Guinea pig safety test.

The guinea pig safety test provided in this section shall be conducted when prescribed in a Standard Requirement or approved Outline of Production for a biological product. When desiccated products are tested, final container samples of completed product prepared for administration in the manner recommended on the label shall be used. When liquid products are tested, either bulk or final container samples of completed product shall be used.

(a) Unless otherwise specified in the Standard Requirement or approved Outline of Production for the product, a 2 ml dose shall be injected either intramuscularly or subcutaneously into each of two guinea pigs and the animals observed for 7 days.

(b) If unfavorable reactions attributable to the product occur in either of the guinea pigs during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[39 FR 16857, May 10, 1974; 39 FR 20368, June 10, 1974]

§ 113.39 Cat safety tests.

The safety tests provided in this section shall be conducted when prescribed in a standard requirement or in the filed Outline of Production for a biological product recommended for use in cats.

(a) The cat safety test provided in this paragraph shall be used when the Master Seed Virus is tested for safety.

(1) The test animals shall be determined to be susceptible to the virus under test as follows:

(i) Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of the virus. Blood samples shall also be drawn and individual serum samples tested for antibody to the virus.

(ii) The cats shall be considered susceptible if swabs are negative for virus isolation and the serums are free of virus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other serum-neutralization test of equal sensitivity.

(iii) When determining susceptibility to a virus which does not lend itself to the methods in paragraphs (a)(1)(i) and (ii) of this section, a method acceptable to Animal and Plant Health Inspection Service shall be used.

(2) Each of at least 10 susceptible cats shall be administered a sample of the Master Seed Virus equivalent to the amount of virus to be used in one cat dose of the vaccine, by the method to be recommended on the label, and the cats observed each day for 14 days.

(3) If unfavorable reactions attributable to the virus occur in any of the cats during the observation period, the Master Seed Virus is unsatisfactory. If unfavorable reactions occur which are