

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]