

during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the biological product, the test shall be declared inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial shall be considered unsatisfactory.

[60 FR 14358, Mar. 17, 1995]

§ 113.41 Calf safety test.

The calf safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* Each of two calves shall be injected with the equivalent of 10 doses of vaccine administered in the manner recommended on the label and observed each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the calves 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 27428, July 29, 1974]

§ 113.42 Detection of lymphocytic choriomeningitis contamination.

The test for detection of lymphocytic choriomeningitis (LCM) virus provided in this section shall be conducted when such a test is prescribed in an applicable Standard Requirement or in a filed Outline of Production. Vaccine virus may be neutralized with specific antiserum when necessary.

(a) Each of at least 10 mice obtained from a source free of LCM shall be injected in the footpad of a hindfoot with 0.02 ml of the material being tested and observed each day for 21 days.

(b) If any of the mice show swelling in the injected footpad or if more than one becomes systemically abnormal, the material being tested is unsatisfactory.

[42 FR 6794, Feb. 4, 1977]

§ 113.43 Detection of chlamydial agents.

The test for chlamydial agents provided in this section shall be conducted when such a test is prescribed in an applicable standard requirement or in a filed Outline of Production.

(a) The yolk sac of 6-day-old chicken embryos shall be injected. Three groups of 10 embryos shall be used sequentially.

(1) The inoculum for each embryo in the first group shall consist of 0.5 ml of a mixture of equal parts of the seed virus with phosphate buffered saline that may contain Streptomycin, Vancomycin, Kanamycin, or a combination thereof. Not more than 2 mg/ml of each antibiotic shall be used.

(2) On the 10th day postinoculation, the yolk sac of viable embryos shall be harvested, pooled, homogenized as a 20 percent suspension in phosphate buffered saline antibiotic diluent, and 0.5 ml of the mixture injected into the second group of chicken embryos. This process shall be repeated for the injection of the third group of embryos using the yolk sacs of viable embryos from the second group.

(3) For each of the three passages, embryo deaths occurring within 48 hours of injection shall be disregarded, except that if more than three such deaths occur at any passage, that passage shall be repeated.

(b) If one or more embryo deaths occur at any passage after 48 hours postinjection, the yolk sacs from each of the dead embryos shall be subcultured into 10 additional embryos. If one or more embryo deaths again occur due to chlamydial agents, the Master Seed Virus is unsatisfactory for use to produce vaccine.

[44 FR 58899, Oct. 12, 1979]

§ 113.44 Swine safety test.

The swine safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* (1) Inject each of two swine of the minimum age for which the product is recommended

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with the equivalent of two doses of bacterial vaccine or 10 doses of viral vaccine.

(2) Administer vaccine in the manner recommended on the label.

(3) Observe swine each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the swine 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.

[48 FR 33476, July 22, 1983]

§ 113.45 Sheep safety test.

The sheep safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* (1) Inject each of two sheep of the minimum age for which the product is recommended with the equivalent of two doses of bacterial vaccine or 10 doses of viral vaccine.

(2) Administer vaccine in the manner recommended on the label.

(3) Observe sheep each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the sheep 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.

[48 FR 33476, July 22, 1983]

§ 113.46 Detection of cytopathogenic and/or hemadsorbing agents.

The tests for detection of cytopathogenic and/or hemadsorbing agents provided in this section shall be conducted when prescribed in an applicable Standard Requirement or in the filed Outline of Production for a product.

(a) *Test for cytopathogenic agents.* One or more monolayers that are at least 6 cm² and at least 7 days from the last subculture shall be tested as provided in this paragraph.

(1) Stain each monolayer with a suitable cytological stain.

(2) Examine the entire area of each stained monolayer for evidence of inclusion bodies, abnormal number of giant cells, or other cytopathology indicative of cell abnormalities attributable to an extraneous agent.

(b) *Test for hemadsorbing agents.* One or more monolayers that are at least 6 cm² and at least 7 days from the last subculture shall be tested as provided in this paragraph.

(1) Wash the monolayer with several changes of phosphate buffered saline.

(2) Add an appropriate volume of a 0.2 percent red blood cell suspension to uniformly cover the surface of the monolayer of cultured cells. Suspensions of washed guinea pig and chicken red blood cells shall be used. These suspensions may be mixed before addition to the monolayer or they may be added separately to individual monolayers.

(3) Incubate the monolayer at 4° C for 30 minutes, wash with phosphate buffered saline, and examine for hemadsorption.

(4) If no hemadsorption is apparent, repeat step (b)(2) of this section and incubate the monolayers at 20-25 °C for 30 minutes, wash with phosphate buffered saline, and examine again for hemadsorption. If desired, separate monolayers may be used for each incubation temperature.

(c) If specific cytopathology or hemadsorption attributable to an extraneous agent is found, the material under test is unsatisfactory and shall not be used to prepare biological products. If an extraneous agent is suspected because of cytopathology or hemadsorption and cannot be eliminated as a possibility by additional testing, the material under test is unsatisfactory.

[50 FR 441, Jan. 4, 1985, as amended at 58 FR 50252, Sept. 27, 1993]