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 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 cm^2 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 cm^2 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]

§ 113.47 Detection of extraneous viruses by the fluorescent antibody technique.

The test for detection of extraneous viruses by the fluorescent antibody technique provided in this section shall be conducted when prescribed in an applicable Standard Requirement or in a filed Outline of Production for a product.

(a) Monolayer cultures of cells (monolayers), at least 7 days after the last subculturing, shall be processed and stained with the appropriate antiviral fluorochrome-conjugated antibody as specified in paragraph (b) of this section.

(1) Three groups of one or more monolayers shall be required for each specific virus prescribed in paragraph (b) of this section.

(i) At the time of the last subculturing, one group of test monolayers shall be inoculated with approximately 100–300 $FAID_{50}$ of the specific virus being tested for as positive controls.

(ii) One group of monolayers shall be the ''material under test.''

(iii) One group of monolayers, that are of the same type of cells as the test monolayers and that have been tested as prescribed in §§ 113.51 or 113.52 (whichever is applicable), shall be prepared as negative controls.

(2) Each group of monolayers shall have a total area of at least 6 cm^2.

(3) Positive control monolayers may be fixed (processed so as to arrest growth and assure attachment of the monolayer to the surface of the vessel in which they are grown) before 7 days after subculturing if fluorescence is enhanced by doing so, *Provided*, That a monolayer of the material under test is also fixed at the same time as the positive control and a monolayer of the material under test is also fixed at least seven days after subculturing. Monolayers that are fixed before 7 days after subculturing shall be stained at the same time as the test monolayers and negative controls fixed at least 7 days after subculturing.

(b) The antiviral fluorochrome-conjugated antibodies to be used shall depend on the type of cells required to be tested for extraneous viruses as specified in an applicable Standard Requirement or in a filed Outline of Production. Antiviral fluorochrome-conjugated antibodies specific for the extraneous viruses shall be applied to each respective type of cell in accordance with the following list. Under certain circumstances, additional tests may need to be conducted, as determined by the Administrator. When a specific antiviral fluorochrome-conjugated antibody is used in testing for the listed extraneous viruses specified in more than one cell type, it need only be applied to the most susceptible cell type.

(1) All cells shall be tested for:

(i) Bovine virus diarrhea virus;

(ii) Reovirus; and

(iii) Rabies virus.

(2) Bovine, caprine, and ovine cells shall, in addition, be tested for:

(i) Bluetongue virus;

(ii) Bovine adenoviruses;

(iii) Bovine parvovirus; and

(iv) Bovine respiratory syncytial virus.

(3) Canine cells shall, in addition, be tested for:

(i) Canine coronavirus;

(ii) Canine distemper virus; and

(iii) Canine parvovirus.

(4) Equine cells shall, in addition, be tested for: