

An inactivated viral product shall be exempt from this requirement if the licensee can demonstrate to Animal and Plant Health Inspection Service that the agent used to inactivate the vaccine virus would also inactivate lymphoid leukosis virus.

(a) Propagation of contaminating lymphoid leukosis viruses, if present, shall be done in chick embryo cell cultures.

(1) Each vaccine virus, cytopathic to chick embryo fibroblast cells, shall be effectively neutralized, inactivated, or separated so that minimal amounts of lymphoid leukosis virus can be propagated on cell culture during the 21-day growth period. If a vaccine virus cannot be effectively neutralized, inactivated, or separated, a sample of another vaccine prepared the same week from material harvested from each source flock (or other sampling procedure acceptable to Animal and Plant Health Inspection Service) used for the preparation of the questionable vaccine virus that cannot be neutralized, inactivated, or separated shall be tested each week during the preparation of such questionable vaccine.

(2) When cell cultures are tested, 5 ml of the final cell suspension as prepared for seeding of production cell cultures shall be used as inoculum. When vaccines are tested, the equivalent of 200 doses of Newcastle disease vaccine or 500 doses of other vaccines for use in poultry, or one dose of vaccine for use in other animals shall be used as inoculum. Control cultures shall be prepared from the same cell suspension as the cultures for testing the vaccine.

(3) Uninoculated chick embryo fibroblast cell cultures shall act as negative controls. One set of chick fibroblast cultures inoculated with subgroup A virus and another set inoculated with subgroup B virus shall act as positive controls, A and B respectively.

(4) The cell cultures shall be propagated at 35–37 °C for at least 21 days. They shall be passed when necessary to maintain viability and samples harvested from each passage shall be tested for group specific antigen.

(b) The microtiter complement-fixation test shall be performed using either the 50 percent or the 100 percent hemolytic end point technique to de-

termine complement unitage. Five 50 percent hemolytic units or two 100 percent hemolytic units of complement shall be used for each test.

(1) All test materials, including positive and negative controls, shall be stored at –60 °C or colder until used in the test. Before use, each sample shall be thawed and frozen three times to disrupt intact cells and release the group specific antigen.

(2) The antiserum used in the microtiter complement-fixation test shall be a standard reagent supplied or approved by the Animal and Plant Health Inspection Service. Four units of antiserum shall be used for each test.

(3) Presence of complement-fixing activity in the harvested samples (from passages) at the 1:4 or higher dilution, in the absence of anticomplementary activity, shall be considered a positive test unless the activity can definitely be established to be caused by something other than lymphoid leukosis virus, subgroups A and/or B. Activity at the 1:2 dilution shall be considered suspicious and the sample further subcultured to determine presence or absence of the group specific antigen.

(4) Biological products or primary cells which are found contaminated with lymphoid leukosis viruses are unsatisfactory. Source flocks from which contaminated material was obtained are also unsatisfactory.

[38 FR 29888, Oct. 30, 1973, as amended at 38 FR 32917, Nov. 29, 1973; 39 FR 21042, June 18, 1974; 56 FR 66784, Dec. 26, 1991]

§ 113.32 Detection of *Brucella* contamination.

The test for detection of *Brucella* contamination 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 for the product.

(a) One ml of the minced tissue used as the source of cells or 1 ml of the extract of the tissue prior to the addition of antibiotics, diluent and stabilizer, shall be inoculated onto each of three tryptose agar plates and incubated in a 10 percent CO₂ atmosphere at a temperature of 35–37 °C for at least 7 days.

(b) If colonies are identified as *Brucella*, the biological product is unsatisfactory.

(c) If colonies suspicious of *Brucella* are observed but cannot be identified as a *Brucella* species, either

(1) The biological product shall be regarded as unsatisfactory and destroyed; or

(2) Further subculture or other procedures shall be carried out until a positive identification can be made.

[38 FR 29888, Oct. 30, 1973]

§ 113.33 Mouse safety tests.

One of the mouse safety tests provided in this section shall be conducted when such test is prescribed in a Standard Requirement or in the filed Outline of Production for a biological product recommended for animals other than poultry: *Provided*, That if the inherent nature of one or more ingredients makes the biological product lethal or toxic for mice but not lethal or toxic for the animals for which it is recommended, the licensee shall demonstrate the safety of such product by an acceptable test written into such Outline of Production.

(a) Final container samples of completed product from live virus vaccines shall be tested for safety using young adult mice in accordance with the test provided in this paragraph.

(1) Vaccine prepared for use as recommended on the label shall be tested by inoculating eight mice intraperitoneally or subcutaneously with 0.5 mL (the inoculation volume may be divided among more than one injection site), and the animals observed for 7 days.

(2) If unfavorable reactions attributable to the product occur in any of the mice 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.

(b) Bulk or final container samples of completed product from liquid products, such as but not limited to antiserums and bacterins, shall be test-

ed for safety in accordance with the test provided in this paragraph.

(1) Unless otherwise prescribed in the Standard Requirement or approved in a filed Outline of Production for the product, a 0.5 ml dose shall be injected intraperitoneally or subcutaneously into eight mice and the animals observed for 7 days.

(2) If unfavorable reactions attributable to the product occur in any of the mice 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.

[38 FR 34727, Dec. 18, 1973, as amended at 39 FR 16857, May 10, 1974; 72 FR 72564, Dec. 21, 2007]

§ 113.34 Detection of hemagglutinating viruses.

The test for detection of hemagglutinating viruses 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) Final container samples of completed product rehydrated as recommended on the label shall be used as inoculum: *Provided*, That poultry vaccines distributed without diluent shall be rehydrated with 30 ml of sterile distilled water per 1,000 doses and used as inoculum. When one or more fractions are to be used in combination with Newcastle Disease Vaccine, test samples shall be collected from bulk suspensions of each prior to mixing with the Newcastle Disease Vaccine.

(b) Each of ten 9- to 10-day-old embryonating eggs from Newcastle disease susceptible flocks shall be inoculated into the allantoic cavity with 0.2 ml of the undiluted inoculum.

(1) Test five uninoculated embryos of the same age and from the same flock as those used for the test as negative controls.

(2) Test an allantoic fluid sample of Newcastle disease virus as a positive control.