

§ 113.32

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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 determine 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. Eight mice shall be inoculated intracerebrally with 0.03 ml and eight mice shall be inoculated intraperitoneally with 0.5 ml. Both groups shall be observed for 7 days.