

§ 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]

§ 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

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100-300 FAID₅₀ 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 cm².

(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:

- (i) Equine herpesvirus; and
- (ii) Equine viral arteritis virus.

(5) Feline cells shall, in addition, be tested for:

- (i) Feline infectious peritonitis virus; and
- (ii) Feline panleukopenia virus.

(6) Porcine cells shall, in addition, be tested for:

- (i) Porcine adenovirus;
- (ii) Porcine parvovirus;
- (iii) transmissible gastroenteritis virus; and
- (iv) Porcine hemagglutinating encephalitis virus.

(7) Firms that do not have rabies virus on premises either for research or production purposes are exempt from having to produce positive rabies virus control monolayers. Fixed positive rabies virus control monolayers will be provided by the National Veterinary Services Laboratories.

(c) After staining, each group of monolayers shall be examined for the presence of specific fluorescence attributable to the presence of extraneous viruses.

(1) If the material under test shows any evidence of specific viral fluorescence, it is unsatisfactory and may not be used; *Provided*, That, if specific fluorescence attributable to the virus being tested for is absent in the positive control monolayers, the test is inconclusive and may be repeated.

(2) If the fluorescence of the monolayers inoculated with the specific virus as positive controls is equivocal, or if the negative monolayers show equivocal fluorescence indicating possible viral contamination, or both, the test shall be declared inconclusive, and may be repeated; *Provided*, That, if the test is not repeated, the material under test shall be regarded as unsatisfactory for use in the production of biologics.

[60 FR 24548, May 9, 1995]

INGREDIENT REQUIREMENTS

§ 113.50 Ingredients of biological products.

All ingredients used in a licensed biological product shall meet accepted standards of purity and quality; shall be sufficiently nontoxic so that the amount present in the recommended dose of the product shall not be toxic to the recipient; and in the combinations used shall not denature the specific substances in the product below the minimum acceptable potency within the dating period when stored at the recommended temperature.

[38 FR 29889, Oct. 30, 1973]

§ 113.51 Requirements for primary cells used for production of biologics.

Primary cells used to prepare biological products shall be derived from normal tissue of healthy animals. When prescribed in an applicable Standard Requirement or in the filed Outline of Production, each batch of primary cells used to prepare a biological product shall be tested as prescribed in this section. A batch of primary cells found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from primary cells that are found unsatisfactory by any prescribed test.

(a) Final container samples of completed product or samples of the final pool of harvested material or samples of each subculture of cells used to prepare the biological product shall be shown free of mycoplasma as prescribed in §113.28. The sample for testing shall consist of at least 75 cm² of actively growing cells or the equivalent in harvest fluids; *Provided*, That all sources of cells in the batch of primary cells are represented.

(b) Final container samples of completed product or samples of the final pool of harvested material or samples of each subculture of cells used to prepare the biological product shall be shown free of bacteria and fungi as prescribed in §113.26 or §113.27 (whichever is applicable).

(c) A monolayer at least 75 cm² from each batch of primary cells or each subculture of primary cells used to pre-

pare a biological product shall be shown free of extraneous agents as prescribed in this paragraph.

(1) The test monolayer shall be maintained using the medium (with additives) and under conditions similar to those used to prepare biological products.

(i) Monolayers of avian origin shall be maintained for at least 14 days and shall be subcultured at least once during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm². The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(ii) Monolayers not of avian origin shall be maintained for at least 28 days and shall be subcultured at least twice during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm². The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(2) Monolayers shall be examined regularly throughout the required maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the batch of primary cells is unsatisfactory.

(3) At the conclusion of the required maintenance period, monolayers shall be tested for:

(i) Cytopathogenic and/or hemadsorbing agents as prescribed in §113.46;

(ii) Extraneous viruses by the fluorescent antibody technique as prescribed in §113.47.

[50 FR 442, Jan. 4, 1985, as amended at 60 FR 24549, May 9, 1995]

§ 113.52 Requirements for cell lines used for production of biologics.

When prescribed in an applicable Standard Requirement or in a filed Outline of Production each cell line used to prepare a biological product shall be tested as prescribed in this section. A cell line found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from a cell line that is found unsatisfactory by any prescribed test.