

§ 113.46

§ 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

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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 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.