

shall meet the minimum area requirement specified in §§113.46 and 113.47 and paragraph (f) of this section.

(3) Monolayers shall be examined regularly throughout the 21-day maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the MCS is unsatisfactory.

(4) At the conclusion of the 21-day maintenance period, monolayers shall be tested for:

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

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

(f) At the conclusion of the 21-day maintenance period provided in paragraph (e) of this section, at least one monolayer of at least 75 cm² shall also be shown free of extraneous agents as prescribed in this paragraph.

(1) Alternately freeze and thaw the monolayer(s) three times. Centrifuge the disrupted cells at no greater than 2,000 x g for no more than 15 minutes to remove cellular debris. Divide the supernatant into equal aliquots and dispense 1.0 ml onto each of at least one monolayer (at least 75 cm²) of:

(i) Vero (African green monkey kidney) cell line;

(ii) Embryonic cells, neonatal cells, or a cell line of the same species of origin as the MCS if different than provided in paragraph (f)(1)(i) of this section;

(iii) Embryonic cells, neonatal cells, or a cell line of the species for which the vaccine is recommended if different than provided in paragraph (f)(1)(ii) of this section; and

(iv) Embryonic cells, neonatal cells, or a cell line of bovine origin if not specified in paragraphs (f)(1)(ii), and (iii) of this section.

(2) The monolayers of cells specified in paragraphs (f)(1)(i), (ii), (iii), and (iv) of this section shall be maintained for at least 14 days after inoculation with the aliquot of disrupted MCS. Monolayers 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.

(3) Monolayers shall be examined regularly throughout the 14-day maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the MCS is unsatisfactory.

(4) At the conclusion of the 14-day maintenance period, monolayers shall be tested for:

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

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

(g) The karyology of cells lines used in the production of biologics shall be examined as follows. A minimum of 50 mitotic cells shall be examined at both the MCS and MCS+n. The modal number in the MCS+n shall not exceed plus or minus 15 percent of the modal number of the MCS. Any marker chromosomes present in the MCS shall persist at the MCS+n. If the modal number exceeds the limits and/or the marker chromosomes do not persist (through the MCS+n passage level), the cell line shall not be used for vaccine production.

(h) If direct or indirect evidence exists that a cell line which is intended for use in the preparation of a vaccine may induce malignancies in the species for which the product is intended, that cell line shall be tested for tumorigenicity/oncogenicity by a method acceptable to APHIS.

[50 FR 442, Jan. 4, 1985; 50 FR 3316, Jan. 24, 1985, as amended at 56 FR 66784, Dec. 26, 1991; 60 FR 24549, May 9, 1995]

§ 113.53 Requirements for ingredients of animal origin used for production of biologics.

Each lot of ingredient of animal origin which is not subjected to heat sterilization or other sterilization methods acceptable to Animal and Plant Health Inspection Service (APHIS), such as, but not limited to serum and albumin, used to prepare a biological product shall be tested as prescribed in this section by the licensee or a laboratory acceptable to VS. Results of all tests shall be recorded by the testing laboratory and made a part of the licensee's

§ 113.54

9 CFR Ch. I (1-1-02 Edition)

records. A lot of ingredient found unsatisfactory by any prescribed test shall not be used to prepare a biological product. A serial of biological product shall not be released if produced using an ingredient that is found unsatisfactory by any prescribed test.

(a) Samples of each lot of ingredient of animal origin which is not subjected to heat sterilization, used to prepare a biological product shall be shown free of mycoplasma by the method prescribed in §113.28.

(b) Samples of each lot of ingredient or animal origin which is not subjected to heat sterilization of other sterilization methods acceptable to APHIS used to prepare a biological product shall be shown free of bacteria and fungi as prescribed in §113.26.

(c) Samples of each lot of ingredient of animal origin, except porcine trypsin, which is not subjected to heat sterilization or other viricidal procedure acceptable to APHIS used in the preparation of biological products shall be tested as prescribed in this paragraph;

(1) Monolayers at least 75 cm² of Vero (African green monkey kidney) cell line and of primary cells or a cell line of the same species of origin as the ingredient shall be used in the test. Cell lines used shall have been found satisfactory when tested as prescribed in §113.52 and primary cells used shall have been found satisfactory when tested as prescribed in §113.51.

(2) At least 3.75 ml or 15 percent of the ingredient shall be used in the growth medium for the preparation of at least 75 cm² test monolayers. The ingredient shall also be used in the growth medium when monolayers are subcultured. If the ingredient being tested is cytotoxic when tested in this manner, other procedures may be used if approved by APHIS.

(3) The test monolayers shall be maintained for at least 21 days.

(4) Cells shall be subcultured at least two times during the maintenance period. All but the last subculture shall result in at least one new monolayer of at least 75 cm². The last subculture shall meet the minimum area requirements specified in §§113.46 and 113.47.

(5) Monolayers shall be examined regularly throughout the 21-day maintenance

period for evidence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the ingredient is unsatisfactory.

(6) At the conclusion of the 21-day maintenance period, monolayers shall be tested for:

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

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

(d) Each lot of porcine trypsin which has not been treated to inactivate porcine parvovirus (PPV) in a manner acceptable to VS shall be tested for PPV as prescribed in this paragraph.

(1) Not less than 5.0 grams of trypsin shall be dissolved in a volume of suitable diluent sufficient to fill a centrifuge angle head. After centrifuging for 1 hour at 80,000 x g, the pellet material shall be reconstituted in distilled water and inoculated into a flask containing 75 cm² of a 30 to 50 percent confluent monolayer culture of primary porcine cells or a porcine cell line of proven equal PPV susceptibility. An additional flask of cells shall be held as a negative control.

(2) The test and control monolayers shall be maintained for at least 14 days and subcultured at least once during the maintenance period.

(3) At the end of the 14-day maintenance period, and 4 to 7 days after the last subculturing, monolayers shall be tested for the presence of porcine parvovirus by the fluorescent antibody technique as prescribed in §113.47(c).

(e) A sample of serum from each donor horse used to produce a lot of equine serum used in the preparation of biological products recommended for use in horses shall be tested at a laboratory approved by Animal and Plant Health Inspection Service using the Coggins test for equine infectious anemia antibodies. If antibodies to equine infectious anemia are found, the lot of serum is unsatisfactory.

[50 FR 442, Jan. 4, 1985; 50 FR 3316, Jan. 24, 1985, as amended at 56 FR 66784, Dec. 26, 1991; 60 FR 24549, May 9, 1995]

§ 113.54 Sterile diluent.

Sterile Diluent shall be supplied in a final container by the licensee when