(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 cm^2. 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 cm^2. 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.

(a) *General requirements.* (1) A complete record of the cell line shall be kept, such as, but not limited to, the source, passage history, and medium used for propagation.

(2) A Master Cell Stock (MCS) shall be established at a specified passage level for each cell line. The passage level and identity of the MCS and the highest passage level (MCS + n) intended for use in the preparation of a biological product shall be specified in the Outline of Production for the product.

(3) Sufficient 1.0 ml or larger aliquots of MCS and MCS + n shall be prepared, kept in a frozen state, and made available to Animal and Plant Health Inspection Service (APHIS) upon request for performing the tests prescribed in this section.

(4) Each lot of cells shall be monitored for the characteristics determined to be normal for the cell line, such as, but not limited to, microscopic appearance, growth rate, acid production, or other observable features.

(b) The MCS shall be shown to be of the same species of origin as that reported in paragraph (a)(1) of this section by the following method:

(1) At least four monolayers with a total area of at least 6 cm^2 shall be grown to at least 80 percent confluency.

(2) The monolayers shall be removed from their media, processed, stained, and examined.

(i) At least two monolayers shall be stained with an antispecies fluorchrome-conjugated antibody unrelated to the species of origin of the MCS.

(ii) At least two monolayers shall be stained with an antispecies fluorochrome-conjugated antibody specific to the species of origin of the MCS.

(iii) All monolayers shall be examined for evidence of specific fluorescence.

(3) If specific fluorescence is not found in the monolayers stained with the conjugate specific to the species of origin of the MCS, the cell line is unsatisfactory and shall not be used for vaccine production.

(4) If nonspecific fluorescence is found in the monolayers stained with conjugate from an unrelated species of origin or other results make the test results equivocal, the procedure shall be repeated until either specific fluorescence is found only in the monolayers stained with conjugate specific to the species of origin of the MCS and not in the control monolayers or

specific fluorescence cannot be identified and the MCS is declared unsatisfactory.

(5) Alternate tests to determine the species of origin of the MCS may be used if approved by APHIS.

(c) The MCS and either each subculture of cells used to prepare a biological product or the final pool of harvested material (with or without the stabilizer) or final container samples of completed product for each serial of such product shall be shown to be free of mycoplasma as prescribed in §113.28. The sample for testing shall consist of at least 75 cm^2 of actively growing cells or the equivalent, in harvest fluids. The cells shall represent all sources of cells in the batch.

(d) The MCS and either each subculture used to prepare a biological product or the final pool of harvested material for each serial of such product or final container samples of completed product for each serial of such product shall be tested for bacteria and fungi as prescribed in §113.26 or §113.27 (whichever is applicable). If bacteria or fungi are found in the MCS, the MCS shall not be used. If bacteria or fungi are found in a subculture, the subculture shall not be used.

(e) A monolayer at least 75 cm^2 from each MCS shall be shown free of extraneous agents as prescribed in this paragraph.

(1) The test monolayer shall be maintained for at least 21 days using the medium (with additives) intended for growth and maintenance and under conditions similar to those used to prepare biological products.

(2) 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 cm^2. The last subculture 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 cm^2 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×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 cm^2) 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 cm^2. 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 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 cm^2 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 cm^2 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 cm^2. 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