§ 630.5 General requirements.

(a) Consistency of manufacture. No lot of final vaccine shall be released unless it is one of a series of five consecutive lots produced by the same manufacturing process, all of which have shown negative results with respect to all tests for the presence of live poliovirus, and unless each of the monovalent pools of which a polyvalent final vaccine is composed similarly is one of a series of five consecutive monovalent pools of the same type of inactivated poliovirus, all of which have shown negative results in all tests for the presence of live poliovirus.

(b) Dose. These additional standards are based on a human dose of 1.0 milliliter for a single injection and a total human immunizing dose of three injections of 1.0 milliliter given at appropriate intervals.

(c) Samples and protocols. For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) A 2,500 milliliter sample, neutralized, not dialyzed, and without final preservative, taken at the latest possible stage of manufacturing before the addition of such preservative.

(2) A 200 milliliter bulk sample of the final vaccine containing final preservative.

(3) A total of not less than a 200 milliliter sample of the final vaccine in final labeled containers.

(4) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.


§ 630.10 Poliovirus Vaccine Live Oral Trivalent.

(a) Proper name and definition. The proper name of this product shall be Poliovirus Vaccine Live Oral Trivalent. The vaccine shall be a preparation containing the three types of live, attenuated polioviruses grown in monkey kidney cell cultures, or in a cell line found by the Director, Center for Biologics Evaluation and Research, to meet the requirements of §610.18(c) of this chapter. The vaccine shall be prepared in a form suitable for oral administration.

(b) Criteria for acceptable strains. (1) The Sabin strains of attenuated poliovirus, Type 1 (LS- c, 2ab/KP1), Type 2 (P712, Ch, 2ab/KP2), and Type 3 (Leon 12a, b/KP3), or derivatives from them, may be used in the manufacture of vaccine.

(2)(i) Other poliovirus strains may be used in the manufacture of Poliovirus Vaccine Live Oral Trivalent provided that they are identified by historical records including:

(A) Origin,
Food and Drug Administration, HHS § 630.10

(B) Techniques of attenuation,  
(C) Antigenic properties,  
(D) Neurovirulence for monkeys,  
(E) Pathogenicity for tissue cultures of various cell types, and  
(F) Established virus markers, including rct/40, and d.  

(ii) The data shall be submitted to the Director, Center for Biologics Evaluation and Research, along with other data that establish:  
(A) That each such strain is at least as safe as the Sabin strain of the corresponding type,  
(B) That each such strain demonstrates results comparable to the Sabin strain when inoculated into monkeys by the intrathalamic and intramuscular routes, and  
(C) That each such strain has been used to produce vaccines meeting the safety and potency requirements of §§630.11, 630.15, 630.16 or 630.17, and 630.18.

(3) The Director, Center for Biologics Evaluation and Research, may prohibit the use of a specified strain whenever the Director finds that it is practicable to use another strain of the same type that will produce a vaccine of greater safety and of at least equivalent potency.

(4) If vaccine lots have been produced directly from strain materials (e.g., Sabin Original, Sabin Original Merck, or Sabin Original Rederived), the strain material is not required to be tested in accordance with the provisions of §630.10(c).

(c) Criteria for qualification of the seed virus.  
(1) Each seed virus used in vaccine manufacture shall be prepared from an acceptable strain in monkey kidney cell cultures, derived from animals which have met all of the requirements of §630.12(a), or in a cell culture of a type determined to be suitable by the Director, Center for Biologics Evaluation and Research. The seed virus used in vaccine manufactures shall be demonstrated to be free of extraneous microbial agents except for unavoidable bacteriophage.

(2) Seed virus used for the manufacture of oral poliovirus vaccine shall meet the requirements of §§630.13, 630.16 or 630.17, and 630.18. In addition, the neurovirulence of each of the first five consecutive monovalent virus pools prepared from the seed virus shall meet the neurovirulence requirements prescribed in §§630.16(b)(2) or 630.17(b)(3).

(3) A new seed virus may be used for production provided data are submitted in the form of a product license a supplement that show the new seed virus and each of the first five consecutive monovalent virus pools prepared from it meet the safety requirements of §§630.13 and 630.16 or 630.17 and 630.18 and approval for the use of the seed virus is received in writing from the Director, Center for Biologics Evaluation and Research.

(4) Seed virus in vaccine manufacture shall be prepared in a seed lot system from a master virus seed lot at a passage level consistent with §630.13(a).

(5) For monovalent virus pools tested in accordance with §630.16(b), the use of the seed virus may continue provided that the frequency of monovalent virus pools produced with it which fail to meet the criteria of neurovirulence for monkeys prescribed in §630.16(b)(2) is not greater than predicted on the basis of comparison with the corresponding reference preparation. If the frequency of monovalent virus pools produced with the same seed virus which fail to meet the criteria of neurovirulence for monkeys prescribed in §§630.16(b)(2) is greater than the predicted 1 percent on the basis of the 99-percent fiduciary one-sided upper limit, that seed virus shall be disqualified for further use in vaccine production.

(6) For monovalent virus pools tested in accordance with §630.17, subsequent and identical neurovirulence tests of the seed virus shall be performed in monkeys whenever there is evidence of a significant increase in the neurovirulence of the seed virus, upon introduction of a new production seed lot, and as often as is necessary to otherwise establish, to the satisfaction of the Director, Center for Biologics Evaluation and Research, that the seed virus for vaccine manufacture has maintained its neurovirulence properties as set forth in §630.17(b)(3).
§ 630.11 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design conducted in compliance with part 56 of this chapter, unless exempted under § 56.104 or granted a waiver under § 56.105, and with part 50 of this chapter. Such clinical trials shall be conducted with five lots of oral poliovirus vaccine that have been manufactured by the same methods. Type specific neutralizing antibody for each type of poliovirus in the vaccine shall be induced in 90 percent or more of susceptibles after a series of doses.

§ 630.12 Animal source and quarantine personnel.

(a) Monkeys—(1) Species permissible as source of kidney tissue. Only Macaca monkeys, Cercopithecus monkeys, or other species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable, which meet the requirements of § 600.11(f)(2) and (f)(8) of this chapter, shall be used as the source of kidney tissue for the manufacture of Poliovirus Vaccine Live Oral Trivalent.

(2) Experimental and test monkeys. Monkeys that have been used previously for experimental or test purposes shall not be used as a source of kidney tissue in the processing of vaccine.

(3) Quarantine; additional requirements. Excluding deaths from accidents or causes not due to infectious diseases, if the death rate of any group of monkeys being conditioned in accordance with § 600.11(f)(2) of this chapter exceeds 5 percent per month, the remaining monkeys may be used for the manufacture of Poliovirus Vaccine Live Oral Trivalent only if all of the monkeys survive a new quarantine period.

(b) Personnel. All reasonably possible steps shall be taken to ensure that personnel involved in processing the vaccine are immune to all three types of poliovirus and do not excrete poliovirus.

[56 FR 21432, May 8, 1991; 56 FR 27787, June 17, 1991]

§ 630.13 Manufacture of Poliovirus Vaccine Live Oral Trivalent.

(a) Virus passages. Virus in the final vaccine shall represent no more than five tissue culture passages from the original strain or no more than five tissue culture passages from a virus clone derived from one of the first five tissue culture passages of the original strain.

(b) Virus propagated in primary monkey kidney cell cultures—(1) Continuous cell lines. When primary monkeys kidney cell cultures are used in the manufacture of poliovirus vaccine, continuous cell lines shall not be introduced or propagated in vaccine manufacturing areas.

(2) Identification of processed kidneys. The kidneys from each monkey shall be processed separately. The resulting viral fluid shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in paragraphs (b)(3) and (b)(4) of this section relating to that pair of kidneys have been withdrawn from the harvest.

(3) Monkey kidney tissue production vessels prior to virus inoculation. Prior to inoculation with the seed virus and at least 3 days after complete formation of the tissue sheet, the tissue culture growth in vessels derived from each pair of kidneys shall be examined microscopically for evidence of cell degeneration. If such evidence is observed, the tissue cultures from that pair of kidneys shall not be used for poliovirus vaccine manufacture. To test the tissue found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of four culture systems:

(i) Macaca monkey kidney cells,

(ii) Cercopithecus monkey kidney cells,

(iii) Primary rabbit kidney cells, and

(iv) Cells from one of the systems described in § 630.18(a)(6).

The fluid shall be tested in the following manner: Aliquots of fluid from each vessel derived from the same pair of kidneys shall be pooled and at least 10 milliliters of the pool inoculated into each system. The dilution of the
pool with medium shall be no greater than 1:4 and the area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. At the end of the observation period, at least one subculture of fluid from the Cercopithecus monkey kidney cell cultures shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. If these tests indicate the presence in the monkey kidney tissue culture production vessels of any viable microbial agent, the viral harvest from these tissue cultures so implicated shall not be used for poliovirus vaccine manufacture. 

(4) Control vessels. At least 25 percent of the cell suspension from each pair of kidneys shall be set aside and used to establish control cultures. The control cultures shall be examined microscopically for cell degeneration for an additional 14 days. The culture fluids from such control cells shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the method prescribed for testing of fluids in paragraph (b)(3) of this section. In addition, the control cell sheet shall be examined for presence of hemadsorbing viruses by the addition of guinea pig red blood cells.

(5) Interpretation of test results. At least 80 percent of the control vessels shall be free of cell degeneration at the end of the observation period to qualify the kidneys for poliovirus vaccine manufacture. If the test results of the control cells indicate the presence of any extraneous agent at the time of virus harvest, the virus harvest from that tissue culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in paragraph (b)(3) or (b)(4) of this section demonstrate the presence in the tissue culture preparation of any microbial agent known to be capable of producing human disease, the virus grown in each tissue culture preparation shall not be used for poliovirus vaccine manufacture.

(6) Temperature of kidney tissue production vessels after virus inoculation. After virus inoculation, production vessels shall be maintained at 33.0 to 35.0 °C during the course of virus propagation.

(7) Kidney tissue virus harvests. Virus shall be harvested not later than 72 hours after virus inoculation. Virus harvested from vessels containing the kidney tissue from one monkey may be tested separately, or samples of viral harvests from more than one pair of kidneys may be combined, identified, and tested as a monovalent virus pool. Each pool shall be mixed thoroughly and samples withdrawn for testing as prescribed in §630.18(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at –60 °C or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(8) Filtration. After harvesting and removal of samples for the safety tests prescribed in §630.18(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

§630.14 Reference virus preparations.

(a) Titration test controls. The following reference viruses may be obtained from the Center for Biologics Evaluation and Research:

(1) Reference Poliovirus, Live, Attenuated, Type 1, as a control for correlation of virus titers in tissue cultures.

(2) Reference Poliovirus, Live, Attenuated, Type 2, as a control for correlation of virus titers in tissue cultures.

(3) Reference Poliovirus, Live, Attenuated, Type 3, as a control for correlation of virus titers in tissue cultures.

(4) Reference Poliovirus, Live, Attenuated, Trivalent, as a control for correlation of virus titers in tissue cultures.

(b) Neurovirulence test controls. (1) Except as provided in paragraph (b)(2) of this section, the following reference virus may be obtained from the Center for Biologics Evaluation and Research:
§ 630.15 Potency test.

(a) Test for virus titer. The concentration of living virus in each monovalent virus pool and in each trivalent vaccine, expressed as infectivity titer per milliliter for cell cultures, shall be determined using the Reference Poliovirus, Live, Attenuated of the same type as a control or using another reference preparation of the same type that has been calibrated against the appropriate reference preparation listed in §630.14(a). A titration of the monovalent virus pool or the trivalent vaccine shall not constitute a valid test unless the titration of the reference virus when tested in parallel is within ±0.5 log₁₀ of its established titer. The titration of the parallel reference is intended to validate the test system and shall not be used to adjust the titer of the pool or lot under test.

(b) Dose. The human dose of trivalent vaccines shall be constituted to have infectivity titers in the final container material of 10⁶.0 to 10⁷.0 for type 1, 10⁵.1 to 10⁶.1 for type 2, and 10⁵.8 to 10⁶.8 for type 3, when assayed in HEp-2 cells, or the equivalent when titrated by a different method.

§ 630.16 Test for neurovirulence.

(a) Except as provided in §630.17, the following test relating to safety prescribed in paragraph (b) of this section shall be performed on each monovalent virus pool after the filtration process.

(b) Neurovirulence in monkeys. Except as provided in paragraph (b)(5) of this section, each monovalent virus pool shall be tested concurrently with the corresponding type Reference Attenuated Poliovirus for neurovirulence by the intraspinal route of injection in Macaca monkeys. Whenever possible the monkeys should be of comparable age and weight and from the same quarantine group. The monkeys shall be distributed randomly between the two test groups. If the number of monkeys included in both groups precludes completion during a single workday, approximately equal numbers of monkeys shall be inoculated with the monovalent virus pool and the reference preparation during each of the testing days. A preinjection serum sample obtained from each monkey shall be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 TCID₅₀ (mean tissue culture infectious doses) of each of the three types of poliovirus. The neurovirulence test is not valid unless the inoculation sample is shown to contain the equivalent of 10⁶.5 to 10⁷.5 TCID₅₀ per milliliter when a representative sample of the monovalent virus pool is titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) Intraspinal inoculation. For tests with type 1 and type 2 monovalent virus pools and the Reference Attenuated Poliovirus of the corresponding types, each of a group of at least 12 monkeys after being suitably anesthetized shall be injected intraspinally into the enlargement of the lumbar cord with 0.1 milliliter of the inoculation sample. For tests with type 3 poliovirus materials, groups of at least 20 monkeys shall be injected as above after being suitably anesthetized. A test of a virus pool shall include at least one group of monkeys, and no more than three groups shall be inoculated, with the results from testing one, two, or three groups of monkeys being evaluated as prescribed in §630.16(b)(2). In addition, if on examination there is no evidence of correct inoculation, additional animals may be inoculated in order to reestablish the minimum number of 11 positive monkeys for tests of types 1 and 2 virus.
pools and the minimum number of 18 positive monkeys for tests of Type 3 virus pools. A positive monkey is an animal which either survives for 11 or more days or succumbs or is sacrificed due to a severe poliovirus infection at any time before the 11th day of the observation period and in which neural lesions specific for poliovirus are seen in the central nervous system. If at least 60 percent of the animals of a group survive 48 hours after inoculation, those animals that did not survive may be replaced by additional animals. If less than 60 percent of the animals in a group survive 48 hours after inoculation, the test shall be considered invalid and shall be repeated.

(2) Determination of neurovirulence. At the conclusion of the observation period, the animals are sacrificed and a comparative evaluation shall be made of the evidence of neurovirulence of the monovalent virus pool under test and the Reference Attenuated Poliovirus of the corresponding type with respect to the histopathology of lesions caused by poliovirus. Animals dying or sacrificed when severely paralyzed or moribund during the test period, should be included in the evaluation, except that these examinations of these monkeys shall be made immediately after death. Histopathological examinations by a qualified pathologist shall be made of at least the lumbar and cervical enlargements, the medulla, the mesencephalon, the thalamus, and motor cortex of each monkey in the groups injected with the monovalent virus pool or with the reference under test. The magnitude of the neuropathology exhibited in the lumbar and cervical areas, the medulla, and mesencephalon of all positive monkeys inoculated with the monovalent virus pool shall be quantified and compared to the magnitude of the neuropathology determined based on the same type of evaluation of monkeys in the current test and all previous tests of the Reference Attenuated Poliovirus of the corresponding type. The monovalent virus pool may be used for poliovirus vaccine if a comparative analysis of the test results demonstrates that the numerical value assigned for neurovirulence of the monovalent virus pool is equal to or less than that of the Reference Attenuated Poliovirus of the corresponding type. If the numerical value assigned for neurovirulence of the monovalent virus pool is greater than that of the Reference Attenuated Poliovirus, the monovalent virus pool is acceptable if the difference is not greater than that calculated by a mathematical method that is expected to reject vaccines with neurovirulence identical to the reference at a frequency of not less than 1 in 100 when 1 group of monkeys is inoculated. If 2 groups are injected with the same monovalent virus pool under test, the frequency of rejection shall be not less than 5 in 100 and for 3 groups, not less than 10 in 100. If the difference in numerical values is greater than that calculated, irrespective of which reference preparation was used in the test, the monovalent virus pool shall be considered unacceptable and shall not be used for vaccine manufacture.

(3) Outlier scores. In the event that one or more monkeys inoculated with virus from the monovalent virus pool have individual mean lesion scores higher than that previously or concurrently associated with the Reference Attenuated Poliovirus of the corresponding type, but the monovalent virus pool meets the criteria for acceptable neurovirulence given in §630.16(b)(2), the significance of the outlier scores shall be evaluated by a method approved by the Director, Center for Biologics Evaluation and Research before the vaccine may be released for use.

(4) Test with Reference Attenuated Poliovirus. Except as provided in paragraph (b)(5) of this section, the Reference Attenuated Poliovirus of the appropriate type shall be tested as prescribed in paragraph (b)(1)(i) of this section concurrently with the monovalent virus pool. More than one monovalent virus pool of the same type may be tested with the same corresponding Reference Attenuated Poliovirus. Initially, a minimum of four tests by the testing laboratory of each Reference Attenuated Poliovirus is required. These tests must be such as to provide sufficient experience to define the performance of the Reference Attenuated Poliovirus and establish the variability of the assay. Each test of
§ 630.17 Alternative test for neurovirulence.

(a) In lieu of the neurovirulence test in §630.16, the following test may be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot).

(b) Neurovirulence in monkeys. Each monovalent virus pool or monovalent lot shall be tested in comparison with the Reference Attenuated Poliovirus, Type 1, for neurovirulence in Macaca monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against not more than 1,000 TCID₅₀ (mean tissue culture infectious dose) of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least 10⁷₆ TCID₅₀ per milliliter when titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) Intrathalamic inoculation. Each of at least 30 monkeys shall be injected intracerebrally by placing 0.5 milliliter of virus pool material into the thalamic region of each hemisphere. Comparative evaluations shall be made with the virus pool under test and the Reference Attenuated Poliovirus, Type 1. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least 24 monkeys are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate virus pool material into the thalamus, additional monkeys may be inoculated in order to reestablish the minimum number of monkeys for the test.

(2) Intraspinal inoculation. Each of a group of at least five monkeys shall be injected intraspinally with 0.2 milliliter of virus pool material containing at least 10⁷₆ TCID₅₀ per milliliter when titrated in HEp-2 cells, and each monkey in additional groups of at least five monkeys shall be injected intraspinally with 0.2 milliliter of a 1:1,000 and 1:10,000 dilution, respectively, of the same virus pool material. Comparative evaluations shall be made with the virus pool under test and the reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least four monkeys per group are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate intraspinally, additional animals may be inoculated in order to reestablish the minimum number of animals per group.

(3) Determination of neurovirulence. At the conclusion of the observation period comparative histopathological examinations by a qualified pathologist shall be made of the lumbar cord, cervical cord, lower medulla, mesencephalon and motor cortex of each monkey in the groups injected with virus under test and those injected with the Reference Attenuated Poliovirus, Type 1, except that for animals dying during the test period, these examinations shall be made immediately after death. If at least 60
percent of the animals of a group survive 48 hours after inoculation, those animals which did not survive may be replaced by an equal number of animals tested as prescribed in paragraph (b) of this section. If less than 60 percent of the animals of a group survive 48 hours after inoculation, the test must be repeated. At the conclusion of the observation the animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristics of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the Reference Attenuated Poliovirus, Type 1, with respect to:

(i) The number of animals showing lesions characteristic of poliovirus infection;
(ii) The number of animals showing lesions other than those characteristic of poliovirus infection;
(iii) The severity of the lesions;
(iv) The degree of dissemination of the lesions; and
(v) The rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. These five factors may be weighted and interpreted as the Director, Center for Biologics Evaluation and Research, or the Director's delegatees deem appropriate. Among permissible interpretations, the factors may be considered in different ways for monkeys inoculated intraspinally and for monkeys inoculated intrathalamically. Other relevant factors in addition to those listed in paragraph (b)(3)(i) through (b)(3)(v) of this section, such as public health consequences, may be considered in evaluating neurovirulence test results. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrates that the neurovirulence of the test virus pool does not exceed that of the Reference Attenuated Poliovirus, Type 1.

(4) Test with Reference Attenuated Poliovirus. The Reference Attenuated Poliovirus, Type 1, shall be tested as prescribed in paragraphs (b)(1) and (b)(2) of this section at least once for every 10 production lots of vaccine, except that the interval between the test of the reference and the test of any lot of vaccine shall not be greater than 3 months. The test procedure shall be considered acceptable only if lesions of poliomyelitis are seen in monkeys inoculated with the reference material at a frequency statistically compatible with all previous tests with this preparation.

§ 630.18 Additional tests for safety.

(a) Tests prior to filtration. Monovalent virus pools shall contain no demonstrable viable microbial agent, except for unavoidable bacteriophage and the intended attenuated live poliovirus. The vaccine shall be tested for the absence of other infectious agents, including polioviruses of other types or strains. Testing of each monovalent pool shall include the following procedures:

(1) Inoculation of rabbits. A minimum of 100 milliliters of each monovalent virus pool shall be tested by inoculation into at least 10 healthy rabbits, each weighing 1,500 to 2,500 grams. Each rabbit shall be injected with a total of 1.0 milliliter intradermally in multiple sites, and subcutaneously with 9.0 milliliters, of the monovalent virus pool and the animals observed for at least 3 weeks. Each rabbit that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and the brain and organs removed and examined. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of cercopithecoid herpesvirus 1 or any other viral infection.

(2) Inoculation of adult mice. Each of at least 20 adult mice, each weighing 15 to 20 grams, shall be inoculated intraperitoneally with 0.5 milliliter and intracerebrally with 0.03 milliliter of each monovalent virus pool. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and
examined for evidence of viral infection by direct observation and sub-inoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of lymphocytic choriomeningitis virus or other viral infection.

(3) Inoculation of suckling mice. Each of at least five suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 milliliter and intraperitoneally with 0.1 milliliter of the monovalent virus pool. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by the intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of all animals remain healthy and survive the observation period and if all the mice used in the test fail to show evidence of infection with Mycobacterium tuberculosis or any viral infection.

(4) Inoculation of monkey kidney tissue cultures. At least 500 doses or 50 milliliters, whichever is a greater volume of virus, taken from either each undiluted monovalent virus pool or, in equal proportions from individual harvests or subpools, shall be tested for simian viruses in Macaca monkey kidney tissue cultures and, in the same volume, in Cercopithecus monkey kidney tissue cultures. A dilution of the virus pool in medium not to exceed 1:4 shall be used. The area of surface growth of the cells shall be at least 3 square centimeters per milliliter of test inoculum. The test poliovirus shall be neutralized by high-titer specific antiserum of nonprimate origin. The immunizing antigens used for the preparation of antiserum shall be grown in a cell line other than the cell line used for testing the vaccine. The cultures shall be observed for at least 14 days. At the end of the observation period at least one subculture of fluid from the Cercopithecus kidney cell culture shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all the tissue cultures fail to show evidence of the presence of simian viruses or any other viral infection.

(6) Inoculation of human cell cultures. At least 500 doses or 50 milliliters, whichever represents a greater volume of virus, taken from either single
monovalent pool or, in equal proportions from individual harvests or subpools, shall be tested for the presence of measles virus in either:

(i) Primary human amnion cells,

(ii) Primary human kidney cells, or

(iii) Any other human or nonhuman cell system of comparable susceptibility to unmodified measles virus.

The virus pool shall be diluted with medium not to exceed 1:4. The area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The test material shall be neutralized with poliovirus antiserum of other than primate origin if the tissue culture cell system used is susceptible to poliovirus. The immunizing antigens used for the preparation of antisera shall be grown in a cell line other than the cell line used for testing the vaccine. The culture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus or any other viral infection.

(7) Inoculation of a rabbit kidney tissue culture. At least 500 milliliters of virus pool, taken from either a single monovalent pool or in equal proportions from individual harvests or subpools, shall be tested in primary rabbit kidney tissue culture preparations for evidence of cercopithecoid herpesvirus 1. The virus pool shall be diluted with medium not to exceed 1:4. The area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The culture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus or any other viral infection.


c (b) Tests for in vitro markers. In addition to the neurovirulence test required by §§ 630.16 or 630.17, the following tests relating to safety shall be performed on each monovalent virus pool after the filtration process. Tests shall be performed on each monovalent virus pool using the marker tests described below or other methods shown to be of comparable value in identification of the attenuated strain. The test results shall demonstrate that the monovalent virus pool under test and the seed virus have substantially the same marker characteristics.

(1) rct/40 Marker. Attenuated strains which grow readily at 40 °C (±0.5 °C) are classified as rct/40 positive (+) in contrast to the rct/40 negative (−) strains, which show an increased growth of at least 100,000 fold at 36 °C over that obtained at 40 °C. Comparative determinations shall be made in suitable culture vessels.

(2) d Marker. Attenuated strains which grow readily at low concentrations of bicarbonate under agar are classified as d positive (+) in contrast to the d negative (−) strains, which exhibit delayed growth under the same conditions. The cultures shall be grown in a 36 °C incubator, in suitable culture vessels in an environment of 5 percent CO₂ in air.

c (c) Final container sterility test. The final container sterility test need not be performed provided aseptic techniques are used in the filling process.

§ 630.19 General requirements.

(a) Vaccine release. No lot of trivalent vaccine shall be released by the manufacturer unless each monovalent virus pool contained therein:

(1) Has been manufactured by the same procedures;

(2) Has met the criteria of neurovirulence for monkeys prescribed in §§ 630.16(b) or 630.17(b);

(3) Has met the criteria of in vitro markers prescribed in §630.18(b); and

(4) Has been released for further manufacturing by the Director, Center for Biologics Evaluation and Research unless, at the Director's discretion, the Director determines that lot release by the Center for Biologics Evaluation and Research is not required. The protocols for all monovalent virus pools produced sequentially from the same seed and tested, in whole or in part, in accordance with §§ 630.16(b) or 630.17(b) shall be submitted to the Director, Center for Biologics Evaluation and Research, whether or not release of the pool for further manufacturing is requested. For monovalent virus pools not tested under §§ 630.16(b) or 630.17(b),
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the manufacturer shall report the reasons for partial manufacture to the Director, Center for Biologics Evaluation and Research.

(b) Labeling. In addition to the items required by other applicable labeling provisions of this chapter, the final container label shall bear a statement indicating that liquid vaccine may not be used for more than 7 days after opening the container. Labeling may include a statement indicating that, for frozen vaccine, a maximum of 10 freeze-thaw cycles is permissible provided the total cumulative duration of thaw does not exceed 24 hours, and provided the temperature does not exceed 8°C during the periods of thaw.

(c) Samples and protocols. For each trivalent lot of vaccine and for each monovalent virus pool, the following materials shall be submitted in accordance with instructions received from the Director, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892.

(1) A protocol that consists of a summary of the history of manufacture of each trivalent lot or monovalent virus pool, including any test results requested by the Director, Center for Biologics Evaluation and Research.

(2) Twenty milliliters of monovalent virus pool before filtration.

(3) Forty milliliters of monovalent virus pool after filtration. The titer of the sample shall be no less than the equivalent of 10^{7.5} TCID_{50} per milliliter when titrated in HEp-2 cells; if the titer is greater than 10^{7.5} TCID_{50} per milliliter, a correspondingly smaller volume may be submitted.

(4) A total of at least 50 single doses or the equivalent thereof of the trivalent vaccine.

(5) When deemed appropriate, the Director, Center for Biologics Evaluation and Research, may require submission of samples or sample volumes other than those specified in paragraphs (c)(2), (c)(3), and (c)(4) of this section.

(d) Public health implications. In interpreting any provision of the regulations governing oral poliovirus vaccine, the agency may consider any potential effect on individual or public health, including effects related to vaccine supply.

(e) Alternative procedures. (1) The Director, Center for Biologics Evaluation and Research, may approve an exception or alternative to any requirement in subpart B of part 630 regarding Poliovirus Vaccine Live Oral. Requests for such exceptions or alternatives should ordinarily be made in writing. However, in limited circumstances such requests may be made orally and permission may be given orally by the Director, Center for Biologics Evaluation and Research. Oral requests and approvals must be followed by written requests and written approvals.

(2) FDA will publish a list of approved alternative procedures and exceptions periodically in the Federal Register.

(f) Status of vaccine in distribution. Poliovirus Vaccine Live Oral released or in distribution prior to May 8, 1991, is deemed to meet the requirements of supart B of part 630.

Subpart C—[Reserved]

Subpart D—Measles Virus Vaccine Live

§ 630.30 Measles Virus Vaccine Live

(a) Proper name and definition. The proper name of this product shall be Measles Virus Vaccine Live, which shall consist of a preparation of live, attenuated, measles virus.

(b) Criteria for acceptable strains of attenuated measles virus. Strains used for the manufacture of Measles Virus Vaccine Live, shall have been shown to be safe and potent in man by field studies with experimental vaccines. The vaccine shall have been demonstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous