§ 630.63 Reference virus.
§ 630.64 Potency test.
§ 630.65 Test for safety.
§ 630.66 General requirements.

Subpart H—Smallpox Vaccine

§ 630.70 Smallpox Vaccine.
§ 630.71 Production.
§ 630.72 Reference vaccine.
§ 630.73 Potency test.
§ 630.74 Tests for safety.
§ 630.75 General requirements.


Source: 38 FR 32068, Nov. 20, 1973, unless otherwise noted.

Cross References: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21–12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails, see parts 124 and 125 of the Domestic Mail Manual, that is incorporated by reference in 39 CFR part 111.

Subpart A—Poliovirus Vaccine Inactivated

§ 630.1 Poliovirus Vaccine Inactivated.

(a) Proper name and definition. The proper name of this product shall be “Poliovirus Vaccine Inactivated” which shall consist of an aqueous preparation of poliovirus types 1, 2, and 3, grown in monkey kidney tissue cultures, inactivated by a suitable method.

(b) Strains of virus. Strains of poliovirus used in the manufacture of vaccine shall be identified by historical records, infectivity tests and immunological methods. Any strain of virus may be used that produces a vaccine meeting the requirements of §§630.2, 630.3, and 630.4, but the Director, Center for Biologics Evaluation and Research may from time to time prohibit the use of any specific strain whenever he finds that it is practicable to use another strain of the same type which is potentially less pathogenic to man and that will produce a vaccine of at least equivalent safety and potency.

(c) Monkeys; species permissible as source of kidney tissue. Only Macaca or Cercopithecus monkeys, or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable, which have met all requirements of §§600.11(f)(2) and 600.11(f)(8) of this chapter shall be used as a source of kidney tissue for the manufacture of Poliovirus Vaccine Inactivated.


§ 630.2 Poliovirus Vaccine Inactivated.

(a) Cultivation of virus. Virus for manufacturing vaccine shall be grown with aseptic techniques in monkey kidney tissue cultures. Suitable antibiotics in the minimum concentration required may be used (§ 610.15(c) of this chapter).

(b) Filtration. Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered or clarified by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(c) Virus titer. The 50 percent endpoint (TCID₅₀) of the virus fluids after filtration shall be 10⁶.₅ or greater as confirmed by comparison in a simultaneous test (using groups of 10 tubes at 1 log steps or groups of 5 tubes at 0.₅ log steps) with a reference virus distributed by the Center for Biologics Evaluation and Research. Acceptable titrations of the reference virus shall not vary more than ±₀.₅ log₁₀ from its labeled titer using 0.₅ milliliter inoculum in tissue culture.

(d) Inactivation of virus. The virus shall be inactivated, as evidenced by the tests described in §630.4, through the use of an agent or method which has been demonstrated to be consistently effective in the hands of the manufacturer in inactivating a series of lots of poliovirus. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. solution of formaldehyde of 1:4000, and the inactivation conducted under controlled conditions of pH and time, at a temperature of 36° to 38° C. Three or more virus titers, suitably spaced to indicate rate of inactivation, shall be determined during the inactivation process. Filtration equivalent to that described in paragraph (b) of this section shall be performed after the estimated baseline time (time at which the 50 percent end-
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Each lot of vaccine shall be subjected to a potency test which permits an estimation of the antigenic capacity of the vaccine. This is done by means of a simultaneous comparison of the serum antibody levels produced in monkeys by the vaccine under test with the antibody level of the reference serum distributed by the Center for Biologics Evaluation and Research. The potency test shall be performed on samples taken after all final processing of the product has been completed, including addition of preservative, except that when the final product contains material having an adjuvant effect an additional test shall be performed with a sample taken before the addition of the adjuvant material. The volume of the test sample for the additional test shall be adjusted to the equivalent volume of Poliovirus Vaccine Inactivated in the final product. The test shall be conducted as follows:

(a) Inoculation of monkeys. A group of 12 or more Macaca monkeys, or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose, shall be used. Animals shall weigh between 4 and 8 pounds and shall be in overt good health. Animals that become ill and remain ill during the course of immunization shall be excluded from the group. The test shall not be valid unless at least 10 animals survive the test period and their preinoculation serum antibody levels are as prescribed in paragraph (d) of this section. The test vaccine shall be given intramuscularly to each monkey in 3 doses at 7-day intervals, each dose to be the recommended individual human dose. Only undiluted vaccine shall be used.

(b) Serum samples. A blood sample shall be taken from each monkey prior to vaccination and then again 7 days after the last injection. Serum shall be separated aseptically, and stored under refrigeration.

(c) Serum-virus neutralization test. The titers of individual monkey serums shall be determined in comparison with the reference serum in tests designed to include controls for all the variables of significance including the following:

(1) Serum toxicity control;
(2) Cell control and cell titration;
(3) Virus titration control (at least 4 tubes for each dilution at 0.5 log steps); and
(4) Serum controls using type-specific serums to identify the type of virus used in the neutralization test.

(d) Interpretation of the test. Animals showing preinoculation titers of 1.4 or over when tested against not more
§ 630.4 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer:

(a) The virus pool—tests prior to inactivation—(1) B virus and Mycobacterium tuberculosis. Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(2) SV-40. Prior to inactivation, the material shall be tested for the presence of SV-40 as follows (or by any other test producing equally reliable results): A sample of at least 5 ml. from the virus harvest or virus pool shall be neutralized by high titer specific antiseraum of other than primate origin. A similar sample from the pool of tissue culture fluids from control vessels representing the tissue from which the virus was prepared may be tested in place of the virus sample. The sample shall be tested in primary cercopithecus tissue cultures or in a cell line demonstrated as at least equally susceptible to SV-40. Each tissue culture system shall be observed for at least 14 days.

(b) Single strain pool tissue culture tests for poliovirus. (1) Before pooling to make the final poliovirus vaccine, during inactivation at 36° to 38° C., two samples of each monovalent bulk strain pool shall be tested for the absence of virus by tissue culture methods, the second sample to be taken at least 3 days after taking the first sample.

(2) Each sample shall be no smaller than the equivalent of 1,500 human doses and shall be subjected to the complete testing process and each test shall be performed on a different monkey kidney tissue culture cell preparation. The test sample for one of these tests may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV-40 and poliovirus. Each sample shall be inoculated into five or more tissue culture bottles of a suitable capacity, the ratio of the vaccine to the nutrient fluid being approximately 1:1 to 1:3, and the area of the surface growth of cells being at least 3 square centimeters per milliliter of sample. The tissue culture bottles shall be observed for at least 14 days.

A first subculture shall be made at the end of 7 days from date of inoculation by planting at least 2 percent of the volume from each original bottle into suitable tissue culture vessels, followed by refeeding.

(4) A second subculture shall be made from each original bottle in the same manner at the end of 14 days from date of inoculation.

(5) Each of the first and second subcultures shall be observed for at least 7 days.

(6) If cytopathogenic effects occur either in the original bottles of the two tests or in the subcultures from them, or if cellular degeneration appears in the original bottles or in the subcultures before degeneration occurs in uninoculated cultures, the pool shall be...
held until the matter is resolved. If active poliovirus is indicated, the strain pool shall not be used for inclusion in a final vaccine unless effectively reprocessed as described in §630.2(e). If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from other than the strain pool being tested.

(c) Trivalent vaccine pool tissue culture test. No less than 1,500 human doses of the trivalent vaccine pool, without final preservative, prepared by pooling the three type pools, each of which has passed all tests prescribed in paragraph (b) of this section, shall be subjected to the complete tissue culture test prescribed in such paragraph (b) in at least two approximately equal tests in separate monkey kidney tissue culture preparations. This test sample may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV-40 and poliovirus.

(d) Trivalent vaccine pool lymphocytic choriomeningitis test. The final vaccine shall be shown to be free of lymphocytic choriomeningitis virus by intracerebral inoculation of the maximum volume tolerated into 10 or more mice which shall be observed daily for at least 21 days and a negative test shall not be valid unless at least eight mice survive this period.

(e) Test in monkeys for active virus. (1) Vaccine from final containers selected at random from each filling of each lot shall be pooled to provide a test sample of at least 400 milliliters representing the various fillings. An equal volume of bulk vaccine may be substituted for test samples from each filling lot provided the procedure has been approved by the Director, Center for Biologics Evaluation and Research.

(2) A total of not less than 20 monkeys shall be inoculated with the test sample. A preinjection serum sample from each monkey must not contain neutralizing antibody against the three poliovirus types detectable in a dilution of 1:4 when tested against not more than 1,000 TCID<sub>50</sub> of virus. At least 80 percent of the test animals representing each filling or each bulk sample must survive the test period without significant weight loss, except that if at least 60 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of test animals. At least 80 percent of the animals used in the test must show microscopic evidence of inoculation trauma in the lumbar region of the spinal cord, and gross or microscopic evidence of inoculation trauma in the thalamic area. If less than 60 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals fail to meet the other criteria prescribed in this section, the test must be repeated.

(3) Vaccines shall be injected by combined intracerebral, intraspinal, and intramuscular routes into Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intracerebral injection shall consist of 0.5 milliliter of test sample into the thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of concentrated test sample into the lumbar spinal cord enlargement, the test sample to be concentrated 100 fold in the ultracentrifuge by a method demonstrated to recover at least 90 percent of the virus particles in the sediment after it has been resuspended in the same lot of unconcentrated test sample. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The monkeys shall be observed for 17 to 19 days and signs suggestive of poliomyelitis shall be recorded.

(4) At the end of the observation period, samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification. Histological sections shall be prepared from both spinal cord enlargements and examined.
§ 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.


Subpart B—Poliovirus Vaccine Live Oral Trivalent

SOURCE: 56 FR 21432, May 8, 1991, unless otherwise noted.

§ 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/KP), Type 2 (P712, Ch, 2ab/KP), and Type 3 (Leon 12a1b/KP), 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,