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§ 630.60 Rubella Virus Vaccine Live

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

(b) Criteria for acceptable strains of attenuated rubella virus. Strains of attenuated rubella virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation and (2) antigenic specificity as rubella virus as demonstrated by tissue culture neutralization tests.

(c) Extraneous agents. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents except for unavoidable bacteriophage.

(d) Field studies with experimental vaccines. (1) Strains used for the manufacture of Rubella Virus Vaccine Live, shall have been shown in field studies with experimental vaccines to be safe and potent in the group of individuals inoculated, which must include at least 10,000 susceptible individuals. Susceptibility shall be shown by the absence of neutralizing or hemagglutination-inhibiting antibodies against rubella virus or by other appropriate methods.

(2) The virus strain used in the field studies shall be propagated in the same cell culture system that will be used in the manufacture of the product.

(3) The field studies shall be so conducted that at least 5,000 of the susceptible individuals must reside in areas where health related statistics are regularly compiled in accordance with procedures such as those used by the National Center for Health Statistics. Data in such form as will identify each inoculated person shall be furnished to the Director, Center for Biologics Evaluation and Research.

(4) Inoculated persons shall be shown not to be contagious for contacts through surveillance of rubella susceptible contacts of the inoculated persons.

(e) Neurovirulence safety test of the virus seed strain in monkeys—(1) The test. A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated rubella virus used in the manufacture of rubella vaccine. For this purpose and to establish consistency of manufacture of the vaccine, vaccine from each of five consecutive lots shall be tested separately in monkeys shown to be serologically negative for rubella virus antibodies in the following manner:

(i) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(ii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 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
§ 630.61 Clinical trials to qualify for license.

To qualify for license, the antigenicity of Rubella Virus Vaccine Live, shall be determined by clinical trials, 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, that follow the procedures prescribed in § 630.31, except that the immunogenic effect shall be demonstrated by establishing that a protective antibody response has occurred in at least 90 percent of each of the five groups of rubella-susceptible individuals, each having received the parenteral administration of a virus vaccine dose not greater than that demonstrated to be safe in field studies.

good health and injected under deep barbiturate anesthesia. 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 intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumbar spinal cord enlargement.

(iii) The monkeys shall be observed for 17-21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(iv) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 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 qualified test animals which are tested pursuant to paragraphs (e)(1)(i) through (iii) of this section. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(v) At the end of the observation period, each surviving animal shall be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if needed pursuant to paragraph (e)(1)(vi) of this section. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vi) Doubtful histopathological findings necessitate (a) examination of a sample of sections from several regions of the brain in question, and (b) attempts at virus recovery from the nervous system tissues previously removed from the animal.

(vii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to the presence of unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) Test results. The rubella virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots: (i) 90 percent of the monkeys survive the observation period, (ii) the histological and other studies produce no evidence of changes in the central nervous system attributable to the presence of unusual neurotropism or replication of the seed virus and (iii) there is no evidence of the presence of extraneous neurotropic agents.

(3) Need for additional neurovirulence safety testing. A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in § 630.62(a), (b) and (d) of this part.

§ 630.62 Production.

(a) Virus cultures. Rubella virus shall be propagated in duck embryo cell cultures, rabbit renal 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.

(b) Virus propagated in duck embryo tissue cell cultures. Embryonated duck eggs used as a source of duck embryo tissue for the propagation of rubella virus shall be derived from flocks certified to be free of avian tuberculosis, the avian leucosis-sarcoma group of viruses, reticuloendotheliosis virus, and other agents pathogenic for ducks. Only ducks so certified and in overt good health and which are maintained in quarantine shall be used as a source of duck embryo tissue used in the propagation of rubella virus. Ducks in the quarantined flock that die shall be necropsied and examined for evidence of significant pathologic lesions. If any such signs, symptoms or other significant pathological lesions observed, tissues from that colony shall not be used in the production of vaccine.

(c) [Reserved]

(d) Virus propagated in rabbit renal tissue cell cultures. Only rabbits in overt good health which have been maintained in quarantine individually caged in vermin-proof quarters for a minimum of 6 months, having had no exposure to other rabbits or animals throughout the quarantine period, or rabbits born to rabbits while so quarantined, provided the progeny have been kept in the same type of quarantine continuously from birth shall be used as a source of kidney tissue. Animals shall be free of antibodies for agents potentially pathogenic for man unless it has been demonstrated in the license application that the tests required by §630.65(c) to be performed on each lot of vaccine are capable of detecting contamination of agents capable of producing such antibodies.

(1) Rabbits used for experimental purposes. Rabbits that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the production of vaccine.

(2) Quarantine and necropsy. Each rabbit shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of rabbits, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, myxomatosis, fibromatosis, rabbit pox, and other diseases indigenous to rabbits. If there are any such signs, symptoms or other significant pathological lesions observed, tissues from that colony shall not be used in the production of vaccine.

(3) Control vessels. Control vessels shall be prepared, observed and tested as prescribed in §630.32(f).

(e) Passage of virus strain in vaccine manufacture. Virus in the final vaccine shall represent no more than five cell culture passages beyond the passage used as the seed strain for the manufacture of the vaccine used to perform the field studies (§630.60(d)), which qualified the manufacturer's vaccine strain for license.

(f) Cell cultures in vaccine production areas. Only the cell cultures used in the propagation of rubella virus vaccine shall be introduced into rubella virus vaccine production areas.

(g) Test samples. Test samples of rubella virus harvests or pools shall be withdrawn and maintained by following the procedures prescribed in §630.32(g).

§ 630.63 Reference virus.

A Reference Rubella Virus, Live, shall be obtained from the Center for Biologics Evaluation and Research as a control for correlation of virus titers.

§ 630.64 Potency test.

The concentration of live rubella virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, using either the Reference Rubella Virus, Live, or a calibrated equivalent strain as a titration control. The concentration of live rubella virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 TCID$_{50}$ of the reference virus per human dose.

§ 630.65 Test for safety.

(a) Tests prior to clarification of vaccine manufactured in duck embryo cell cultures. Prior to clarification, the following tests shall be performed on each rubella virus pool prepared in duck embryo cell cultures:

(1) Inoculation of adult mice. The test shall be performed in the volume and following the procedures prescribed in §630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) Inoculation of suckling mice. The test shall be performed in the volume and following the procedures prescribed in §630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) Inoculation of monkey tissue cell cultures. A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in §630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) Inoculation of other cell cultures. The rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in §630.35(a),(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(5) Inoculation of embryonated chicken eggs. A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in §630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) Inoculation of embryonated duck eggs. A suspension of each undiluted rubella virus pool shall be tested in embryonated duck eggs, following the procedures prescribed in §630.35(a)(5), except that the virus need not be neutralized by antiserum and the volume of inoculum per egg shall not exceed 1.0 milliliter. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(7) Bacteriological tests. In addition to the tests for sterility required pursuant to §610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of M. tuberculosis, both avian and human, by appropriate culture methods. The virus pool is satisfactory only if found negative for M. tuberculosis, both avian and human.

(8) Test for avian leucosis. The vaccine shall be tested for avian leucosis, in the volume and following the procedures prescribed in §630.35(a)(8). The cultures are satisfactory for vaccine manufacture if found negative for avian leucosis.

(9) Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum. Each of the tests prescribed in paragraphs (a)(3), (4), (5), and (6) of this section shall be carried out also with rubella virus that has been neutralized by the addition of high titer antiserum of nonhuman, nonsimian and nonavian origin except that the volume of virus suspension of each undiluted virus pool tested shall be no less than 5 ml. The rubella antiserum shall have been prepared by using a rubella virus propagated in a cell culture system other than that used for the manufacture of the vaccine under test, and the cell culture system shall be free of extraneous agents which might elicit antibodies that could inhibit growth of any known extraneous agents which might be present in the vaccine under test. These tests may be performed before or after clarification of the virus. The virus pool is satisfactory only if the results obtained are
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equivalent to those required in those subparagraphs.

(b) [Reserved]

(c) Tests prior to clarification of vaccine manufactured in rabbit renal cell cultures. Prior to clarification each rubella virus pool prepared in rabbit renal cell cultures shall be tested as follows:

(1) Inoculation of adult mice. The test shall be performed in the volume and following the procedures prescribed in §630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) Inoculation of suckling mice. The test shall be performed in the volume and following the procedures prescribed in §630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) Inoculation of monkey tissue cell cultures. A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in §630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) Inoculation of other cell cultures. The tests shall be performed in the volume and following the procedures prescribed in §630.35(a)(3) in rhesus or cynomolgus monkey kidney tissue, rabbit renal tissue and human tissue cell cultures, except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(5) Inoculation of embryonated chicken eggs. A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in §630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) Inoculation of rabbits. A minimum of 15 ml. of each virus pool shall be tested by inoculation into at least five healthy rabbits, each weighing 1500-2500 grams. Each rabbit shall be injected intradermally in multiple sites with a total of 1.0 ml. and subcutaneously with 2.0 ml. of the virus pool, and the animals observed for at least 30 days. 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 virus pool is satisfactory 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 any viral infection.

(7) Inoculation of guinea pigs. Each of at least five guinea pigs, each weighing 350-450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5 ml. of the undiluted virus pool. The animals shall be observed for at least 42 days. Each animal that dies after the first 24 hours of the test or is sacrificed because of illness, shall be necropsied. All remaining animals shall be sacrificed and necropsied at the end of the observation period. The virus pool is satisfactory only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with M. tuberculosis or any viral infection.

(8) Bacteriological tests. In addition to the tests for sterility required pursuant to §610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of M. tuberculosis, human, by appropriate culture methods. The rubella virus pool is satisfactory only if found negative for M. tuberculosis, human.

(9) Tests for adventitious agents. Each virus pool shall be tested for the presence of such known adventitious agents of rabbits as toxoplasma, encephalitozoon, herpes cuniculi, the vacuolating virus of rabbits, rabbit syncytial virus, myxoviruses and reoviruses. The virus pool is satisfactory only if the results of all tests show no evidence of any extraneous agent attributable to the rabbit renal tissue or the vaccine.

(10) Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum. Each of the tests prescribed in paragraphs (c)(3), (4), and (5) of this section shall be carried out also with rubella virus that has been neutralized by the addition of
§ 630.66 General requirements.

(a) Final container tests. In addition to the tests required pursuant to §610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or on the bulk vaccine prior to filling.

(b) Dose. These standards are based on an individual human immunizing dose of no less than 1,000 TCID$_{50}$ of Rubella Virus Vaccine Live, expressed in terms of the assigned titer of the Reference Rubella Virus, Live.

(c) Labeling. In addition to the items required by other applicable labeling provisions of this subchapter, single dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to light.

(d) Photochemical deterioration; protection. Rubella Virus Vaccine Live, in multiple dose containers, shall be protected against photochemical deterioration in accordance with the procedures prescribed in §630.36(g).

(e) Samples; protocols; official release. The following shall be submitted to the Director, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, Md 20892:

(1) For each lot of vaccine:

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

(ii) A total of no less than two 25-milliliter volumes, in a frozen state (−60°C), of preclarification bulk vaccine containing no preservative or adjuvant.

(iii) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

(2) In addition to the requirements of paragraph (e)(1) of this section, whenever a new production seed lot is introduced, or whenever the source of cell culture substrate must be reestablished and recertified, samples consisting of no less than 100 milliliters in 10-milliliter volumes, in a frozen state (−60°C), of postclarification bulk vaccine containing stabilizer but no preservative or adjuvant, taken from each of 5 consecutive lots of the bulk vaccine.

(3) The product shall not be issued by the manufacturer until written notification of official release of the lot is received from the Director, Center for Biologics Evaluation and Research.

§ 630.70 Smallpox Vaccine.

(a) Proper name and definition. The proper name of this product shall be Smallpox Vaccine, which shall be a preparation of live vaccinia virus obtained from inoculated calves or chicken embryos.

(b) Strains of virus. The strain of seed virus used in the manufacture of Smallpox Vaccine shall be identified by historical records including origin and manipulation, and shall meet the sterility test requirements when tested by the procedure prescribed in §610.12 of this chapter. The strain of seed virus and every third passage shall be tested by a rabbit scarification procedure and shown to maintain its original dermatropic properties. The test procedure is available upon request from the Director, Center for Biologics Evaluation and Research. Any new strain shall be shown not to produce a