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(2) A sample of no less than 40 milliliters of the final product distributed in approximately equal amounts into four final containers.

(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.


Subpart D—Cholera Vaccine

§ 620.30 Cholera Vaccine.

The proper name of this product shall be Cholera Vaccine, which shall consist of an aqueous preparation of equal parts of Ogawa and Inaba serotypes of killed Vibrio cholerae bacteria.

[41 FR 18295, May 3, 1976]

§ 620.31 Production.

(a) Strains of bacteria. (1) A strain of Ogawa and a strain of Inaba serotypes of V. cholerae shall be used in the manufacture of the vaccine. Each serotype strain shall have been shown in controlled field studies to yield a vaccine no less potent than vaccines prepared from Ogawa strain 41 and Inaba strain 35A3 obtained from the Center for Biologics Evaluation and Research.

(2) Antigenic integrity of the strains shall be verified by (i) the agglutination of living bacteria of each serotype by cholera O Group I antiserum; (ii) the agglutination of the Ogawa strain in monospecific Ogawa antiserum and of the Inaba strain in monospecific Inaba antiserum; and (iii) the absence of spontaneous agglutination of living bacteria of either strain in 0.85 percent sodium chloride solution during incubation for at least 5 hours at 37°C.

(b) Propagation of bacteria. The culture medium for the propagation strains shall not contain ingredients known to be capable of producing allergic effects in human subjects. The harvested bacteria shall be free of extraneous bacteria, fungi, and yeasts as demonstrated by microscopic examination and cultural methods. Bacteria of the two serotypes shall be grown separately.

(c) Bacterial content. (1) The number of bacteria in each separate bacterial harvest shall be determined by use of the U.S. Opacity Standard not later than 2 hours after harvest and before treatment with a preservative or other agent capable of altering opacity of the bacterial suspension.

(2) The vaccine shall contain equal numbers of bacteria of the Ogawa and Inaba serotypes, and the total number shall not exceed $8 \times 10^9$ bacteria per milliliter.

(d) Nitrogen content. The total nitrogen content of the vaccine shall not exceed 0.3 milligram per milliliter for bacteria grown on solid medium or 1.0 milligram per milliliter if grown in liquid medium. In no instance shall the vaccine contain more than 0.07 milligram per milliliter of nitrogen precipitable by the addition of an equal volume of 10 percent trichloracetic acid.

(e) Preservative. The vaccine shall contain a preservative.


§ 620.32 U.S. Standard preparations.

The following U.S. Standard preparations shall be obtained from the Center for Biologics Evaluation and Research, Food and Drug Administration, for use as prescribed in this subpart:

(a) Vaccine standard. The U.S. Standard Cholera Vaccine, Ogawa serotype, and U.S. Standard Cholera Vaccine, Inaba serotype, shall be reconstituted as directed for determining the potency of Cholera Vaccine.

(b) Opacity standard. The U.S. Opacity Standard for use in estimating the bacterial content of the vaccine and of the challenge culture.

(c) Seed culture. Seed cultures of V. cholerae, Inaba serotype, strain 35A3 and Ogawa serotype, strain 41, for preparation of vaccine challenge cultures for use in the vaccine potency test.


§ 620.33 Potency tests.

Each lot of vaccine shall be subjected to two potency tests. One test shall determine the potency of the vaccine in comparison with the U.S. Standard
Cholera Vaccine, Ogawa serotype, and the other test shall determine the potency of the vaccine in comparison with the U.S. Standard Cholera Vaccine, Inaba serotype. At least four dilutions of each vaccine shall be tested. Each test shall be performed as follows:

(a) **Mice.** Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weights between 10 and 14 grams. A group of at least 16 mice shall be used for each dilution of each vaccine. In addition, there shall be at least 4 groups consisting of no less than 10 mice each for each potency test as a control for virulence titration of the challenge suspension.

(b) **Injections of vaccine.** Serial dilutions, no greater than fivefold, of the vaccine to be tested and of the appropriate serotype standard vaccine shall be made in 0.85 percent sodium chloride solution. The median effective dose (ED$_{50}$), which is the dose of vaccine that is expected to protect 50 percent of the animals that received the vaccine, shall be bracketed by the dilutions used. Each mouse in each dilution group shall receive intraperitoneally 0.5 milliliter of the appropriate vaccine dilution. At least 87.5 percent of the mice in each dilution group shall survive, and all surviving mice shall appear healthy at the time of challenge.

(c) **The challenge.** The challenge shall be administered 12 to 16 days after injection of the vaccine.

(1) The strains of *V. cholerae* for challenge shall be Ogawa 41 and Inaba 35A3, except that *V. cholerae*, Inaba serotype, strain V86 may be used instead of Inaba serotype, strain 35A3, for preparation of vaccine challenge culture: Provided, That the source of the challenge culture shall be identified and verified by the manufacturer as equal to that distributed by the World Health Organization. For each test, the challenge culture shall be taken from a batch of cultures maintained by a method such as freeze-drying that retains constancy of virulence.

(2) The challenge and virulence titration doses shall be prepared as follows: The bacteria for each challenge shall be harvested from a 6- to 18-hour culture grown at 36±1°C, on a suitable agar medium adjusted to pH 7.4. The harvested bacteria shall be uniformly suspended in a diluent consisting of M/15 phosphate buffered saline adjusted to pH 7.4 and shall contain 0.1 to 0.2 percent gelatin. The suspension shall be free from agar particles and clumps of bacteria. The suspension shall be adjusted to an opacity of 10 units, and diluted in tenfold increments using the same diluent. The suspensions for the challenge and virulence titrations shall be suspended in a 5 to 10 percent sterile gastric mucin preparation adjusted to pH 7.4. The challenge suspension shall be prepared from whichever bacterial dilution provides the required median lethal dose (LD$_{50}$) for a 0.5 milliliter challenge dose. The LD$_{50}$ is the dose of the challenge suspension that is expected to kill 50 percent of the animals that received the challenge. The virulence titration suspensions shall consist of the challenge suspension and at least three dilutions of the challenge suspension calculated to bracket the LD$_{50}$ value.

(3) At least 16 surviving mice, randomly selected from each dilution group that received vaccine, shall be inoculated intraperitoneally with a 0.5-milliliter dose of the challenge suspension. Mice in each of the four groups of control mice used for the virulence titration of the challenge suspension shall be inoculated intraperitoneally with a 0.5-milliliter dose of the challenge suspension and its respective dilutions. The challenge dose control mice shall be inoculated last. The interval between removal of the bacteria from the culture medium and the inoculation of the last mouse shall not exceed 2½ hours.

(d) **Recording the results.** The mice shall be observed daily for 2 days following challenge. A daily record shall be maintained of the number of mice that die. A record of the number of mice that survive shall be made at the end of the observation period.

(e) **Validity of the test.** The test is valid provided: (1) The ED$_{50}$ value of the vaccine under test and the standard vaccine is between the largest and smallest doses inoculated into the mice;
§ 620.34 Mouse toxicity test.

The final vaccine shall be demonstrated to be free from toxicity by the following test: A group of no less than 10 and no more than 40 mice, each mouse weighing 14 to 16 grams, shall have free access to food and water at least 2 hours before injection and throughout the test period. The group weight of the mice shall be determined immediately before injection. Each mouse shall be injected intraperitoneally with a test dose of 0.5 milliliter of undiluted vaccine. The group weight of the mice shall be determined again at the end of 72 hours. The 72-hour average weight per mouse shall be no less than the average weight per mouse immediately preceding the injection. No more than 5 percent of the total number of mice used may die during the test period; however, neither death nor significant toxic signs attributable to the vaccine shall result.

[41 FR 18295, May 3, 1976]

§ 620.35 General requirements.

(a) Freezing prohibition. Cholera Vaccine shall not be frozen at any time.

(b) Dose. These standards are based on a total immunizing dose of two injections of 0.5 milliliter and 1.0 milliliter, respectively, given at intervals specified in the manufacturer’s labeling.

(c) Date of manufacture. The date of manufacture shall be the date of initiation of the last valid test for the Ogawa serotype or the Inaba serotype, whichever date is earlier.

(d) Labeling. In addition to the applicable labeling provisions of this chapter, the package label shall bear the following:

(1) A statement that the vaccine contains 8 units of each serotype antigen per milliliter.

(2) The statement, “DO NOT FREEZE”.

(3) The statement, “SHAKE WELL”.

(e) Samples; protocols; official release. 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 sample consisting of no less than 40 milliliters of the product. The sample may be in the final container or from the vaccine bulk lot.

(2) 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,
§ 620.40 BCG Vaccine.

(a) Proper name and definition. The proper name of this product is BCG Vaccine. The product is defined as a freeze-dried preparation containing viable bacteria of the Bacillus of Calmette and Guerin, which is an attenuated strain of Mycobacterium bovis.

(b) Criteria for an acceptable strain. The source of the BCG strain used in the manufacture of any lot of the final product must be identified by complete historical records.

(1) Seed lot system. The BCG strain must be maintained in the form of a primary seed lot that is to be the basic material from which all secondary seed lots are prepared. Production of BCG Vaccine may be from either primary or secondary seed lots. Each seed lot must be stored in either a freeze-dried state at \(-20^\circ C\) or colder, or in a frozen state at \(-70^\circ C\) or colder.

(2) Freedom from virulence. The BCG strain is demonstrated to be incapable of producing progressive tuberculosis in guinea pigs tested as prescribed in §620.45, except that no fewer than 48 guinea pigs must be used to test the primary seed lot and no fewer than 12 guinea pigs must be used to test each secondary seed lot. At least two-thirds of the animals must survive the observation period of no less than 6 months.

(3) Induction of tuberculin sensitivity in guinea pigs. Each of at least 10 guinea pigs is to be injected with 1 human dose of BCG Vaccine and, within 4 to 6 weeks after vaccination, skin tested with tuberculin. At least 80 percent of the guinea pigs tested must develop tuberculin sensitivity, as prescribed in §620.44(b)(3)(ii).

(4) Clinical information. Clinical data must establish that the BCG strain is safe and induces tuberculin sensitivity. After having passed all laboratory tests prescribed for BCG Vaccine, each primary and secondary seed lot of vaccine must be tested for its ability to induce sensitivity in tuberculin-negative persons. Only those persons tested by injection of 5 U.S. Tuberculin Units, Purified Protein Derivative, by the Mantoux technique and found negative in this test are to be selected for clinical trials. At least 100 tuberculin-negative persons must be included in the test of the primary seed lot, and at least 20 tuberculin-negative persons must be included in the test of each secondary seed lot. Within 6 to 8 weeks after BCG vaccination, the vaccinees must be tested for tuberculin reactivity by injecting not more than 10 U.S. Tuberculin Units, Purified Protein Derivate, by the Mantoux technique. The test is considered satisfactory if at least 90 percent of those persons from each group develop tuberculin reactivity as indicated by an induration reaction of at least 5 millimeters in diameter.

§ 620.41 Establishment and personnel requirements.

In addition to the applicable requirements of §§600.10 and 600.11 of this chapter, the following practices and procedures are required:

(a) Isolation of BCG unit. (1) A BCG unit is defined as the space used for storage of primary and secondary seed cultures and for vaccine preparation, including culture maintenance, media inoculation for propagation, harvesting, filling into final containers, sealing of final containers, media production, and cleaning and sterilization of glassware. For purposes of these additional standards, the space used for incubation of bulk and final container sterility tests, tests to determine the numbers of colony-forming units, animal tests, and necropsies are not part of the BCG unit.

(2) The BCG unit must be completely isolated from other production and surrounding areas and must be situated...